Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 40. FEBRUARY, 1939. No. 2.

SECTION MEETINGS

| | | |
|---|---|---|
| **ILLINOIS** | | |
| University of Illinois Medical School | January | 24, 1939 |
| **IOWA** | | |
| State University of Iowa | February | 2, 1939 |
| **MINNESOTA** | | |
| University of Minnesota | January | 18, 1939 |
| **SOUTHERN** | | |
| Tulane University | January | 31, 1939 |
| **SOUTHERN CALIFORNIA** | | |
| California Institute of Technology | December | 28, 1938 |

10336

## Analyses of Gastric Mucosa and Pancreatic Gland Tissue of Dog for $H_2O$, Na, K, Cl and $PO_4$.

R. C. INGRAHAM AND M. B. VISSCHER.

*From the Departments of Physiology, University of Illinois, Chicago, and University of Minnesota, Minneapolis.*

In conjunction with some experiments previously reported by us[1] on gastric and pancreatic secretion, studies were made of the inorganic composition of the gastric mucosa and the pancreas of 12 dogs. The animals were anesthetized with amytal or ether and the organs removed during life. The stomach was carefully washed with water and blotted dry, and the gastric mucosa was peeled off from the muscularis. The pancreas was dissected free from connective tissue and a sample taken. Five gram quantities of each were

1 Ingraham, R. C., and Visscher, M. B., *J. Gen. Physiol.*, 1935, **18**, 695.

used for analysis. The tissues were dried to constant weight at 105°C. Separate samples were wet ashed with superoxyl for the several analyses. Sodium was determined after removal of $PO_4$ by $Ca(OH)_2$ by the Barber and Kolthoff[2] method, potassium by the Breh and Gaebler,[3] chloride by the Van Slyke,[4] and total phosphate by the Fiske and Subbarow[5] methods. The collected data are shown in Table I.

TABLE I.
Summary of Analyses of Gastric Mucosa and Pancreas.

| | Gastric Mucosa | | | | | Pancreas | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $H_2O$ % | Na % Dry wt. | K % Dry wt. | Cl % Dry wt. | $PO_4$ % as P Dry wt. | $H_2O$ % | Na % Dry wt. | K % Dry wt. | Cl % Dry wt. | $PO_4$ % as P Dry wt. |
| 1 | 79.8 | 1.362 | | | 1.037 | 74.2 | 0.499 | | | 1.278 |
| 2 | 79.9 | 1.120 | | | 1.146 | 72.5 | 0.507 | | | 1.391 |
| 3 | 80.0 | 1.471 | | | 1.323 | 74.3 | 0.592 | | | 1.827 |
| 4 | 78.9 | 1.060 | | | 1.019 | 73.6 | 0.601 | | | 1.271 |
| 5 | 81.2 | .992 | | | 1.216 | 71.2 | 0.522 | | | 1.171 |
| 6 | 80.6 | 1.011 | | | 1.004 | 73.7 | 0.512 | | | 1.326 |
| 7 | 80.8 | 1.011 | 0.901 | 1.021 | | 73.8 | 0.599 | 1.068 | 0.659 | |
| 8 | 80.4 | 1.020 | 1.064 | 1.209 | | 74.6 | 0.580 | 1.389 | 0.848 | |
| 9 | 81.8 | 0.988 | 1.092 | 1.088 | | | | | | |
| 10 | 80.3 | 0.876 | 1.026 | 0.949 | | 75.0 | 0.572 | 1.610 | 0.305 | |
| 11 | 80.7 | 0.794 | 1.227 | 0.940 | | 75.8 | 0.529 | 1.426 | 0.568 | |
| 12 | 81.6 | 1.087 | 1.120 | 1.112 | | 76.5 | 0.455 | 2.005 | 0.500 | |
| Mean values: | | | | | | | | | | |
| Gastric Mucosa | | | | | | 80.5 | 1.066 | 1.072 | 1.053 | 1.124 |
| Pancreas | | | | | | 74.1 | 0.543 | 1.500 | 0.560 | 1.377 |

Several important and large differences between the two tissues are evident. The gastric mucosa contains significantly more water, sodium and chloride than the pancreas. In the cases of these 3 components, there was no overlapping of values in the 2 tissues, and there can be little doubt about the significance of the differences. The pancreas contained in every instance more potassium than did the gastric mucosa of the same animal, but there was some overlapping of values between individuals. There was no significant difference in phosphate content between the two tissues.

It is interesting to note that no regular correlation exists between the composition of the tissues and their secreting functions. The gastric glands secrete chloride and contain a higher general percentage of this element than does the pancreas. The pancreas, however, is a sodium secreting organ, but contains less of this element in absolute

[2] Barber, H. H., and Kolthoff, I. M., *J. Am. Chem. Soc.*, 1928, **50**, 1625.

[3] Breh, F., and Gaebler, O. H., *J. Biol. Chem.*, 1930, **87**, 81.

[4] Van Slyke, D. D., *J. Biol. Chem.*, 1923, **58**, 523.

[5] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

percentage than does gastric mucosa, although the ratio of sodium to chloride is greater in the pancreas. Correlations of gland composition and secretory activity must be made with great caution, because it is very likely that the inorganic components of the secretions are derived directly from the blood.

The tissues from animals 8, 9, and 10, were taken after intense histamine and secretin stimulation of the stomach and pancreas, while the remainder were from untreated animals. There are no obvious differences in values obtained from the stimulated glands as compared with the controls. In view of the inherent variability, it is, of course, not impossible, although it seems unlikely, that a larger series might demonstrate a difference. It is obvious, however, that whatever alterations might occur must be relatively small because the sodium figures are rather constant in pancreas and the chloride in gastric mucosa.

These analytical data are presented mainly to show the range of figures found in normal dogs, and to call attention to the differences in composition of the 2 glandular structures, since we were able to find no satisfactory comparative analyses in the literature. Nencke and Simonowski,[6] Guntzner,[7] and Juchler[8] have analyzed for water and chloride. The first named workers found the gastric mucosa to be richer in chloride than the pancreas, in which we confirm them, but their absolute values are much lower than ours for both tissues, and we believe them to be in error. The absolute figures given by the two other workers are nearer to those obtained in this study but those investigators worked with only one tissue. There may be other published values for the composition of these tissues in papers whose titles do not indicate such studies.

*Summary.* Analyses have been made of the water, sodium, potassium and phosphate content of gastric mucosa and pancreas. The results are presented from analyses of tissues from 12 animals.

---

[6] Nencke, M., and Schoumaro Simonowski, E. O., *Arch. f. exp. Path. u. Pharm.*, 1874, **34**, 313.

[7] Grützner, P., *Neue Untersuchungen uber die Bildung und Auscheidung des Pepsins*, Breslau, 1875.

[8] Juchler, Theo., *Z. f. Biochem. u. Biophys.*, 1912, **14**, 347.

10337 P

## Normal Duration of the Q-T Interval.

RICHARD ASHMAN.

*From the Department of Physiology, the Louisiana State University School of Medicine.*

The relation between the average heart cycle length and the Q-T interval was studied on 432 men, 425 women and about 200 children. With exceptions to be given in the final report, these subjects were free from heart disease. In addition, the principal finding for the normal subjects has been confirmed from the study, being continued, of 700 adults and about 200 children with heart disease.

The subjects were subdivided into groups according to sex and age. The principal groups are the younger adult males, the younger adult females, the older males, the older females, and the children. The adult subjects with heart disease were separated according to sex. In every group, without exception, the averages of the Q-T intervals at each cycle length fall closely along a curve represented by the empirical formula, $Q\text{-}T = K \log [10(C + k)]$. C represents the cycle length in seconds, while K and k are constants. For the younger male adults, K was 0.373, and for the females, 0.385. K for all the normal children was 0.376, but became 0.375 when girls, aged 12 to 14 inclusive, were eliminated. For elderly men, K is approximately 0.380, and for women, 0.390. The other constant k, is +0.07 for women, and is very close to, if not precisely at, this value for the other groups. For all groups with heart disease, the value of k remains substantially as given, whereas the value of K rises.

The cube root, square root and straight line formulæ proposed by Fridericia,[1] by Bazett[2] and by Adams,[3] respectively, are inexact. Bazett's formula, using constant 0.39 or 0.40, gives values which are appreciably too low at short cycle lengths and too high at the long cycles. Fridericia's formula, using a constant of 7.95 proposed by Schlomka and Raab[4] for young adults, gives values which are much too high when the heart is rapid and too low when the heart is slow. Adams' formula is still less applicable.

---

1 Fridericia, L. S., *Acta Med. Scand.*, 1920, **53**, 469.

2 Bazett, H. C., *Heart*, 1920, **7**, 353.

3 Adams, W., *J. Clin. Invest.*, 1936, **15**, 335.

4 Schlomka and Raab, *Z. Kreislauft*, 1936, **28**.

10338 P

## Survival of Deciduomata During Lactation in the Rat.

ROBERT A. LYON. (Introduced by Charles Armstrong.)
*From the National Institute of Health, United States Public Health Service, Washington, D. C.*

Deciduomas may be produced in the lactating rat following irritation of the endometrium until the seventeenth day postpartum.[1] This endometrial sensitivity is possible inasmuch as in the rat ovulation occurs a few hours after parturition with resultant functional corpora lutea. The corpora elaborate progestin which together with a small quantity of estrin make sensitization to decidual formation possible.[2] During pregnancy in the rat this sensitization of the endometrium is not prolonged[3] even though the corpora are functional until term. Sensitivity persists longer during lactation than during pregnancy.[4] Probably the estrogen level is considerably lower during lactation than during pregnancy inasmuch as mucification of the vagina is not notable while nursing. Also large quantities of estrogens given soon after ovulation inhibit sensitization.

With these considerations, the purpose of this study is to determine the duration of these growths produced at graded intervals through the lactation period. An understanding of the conditions pertaining to their survival is necessary to aid in the endocrinal interpretation of these and projected studies.

The existence of pregnancy was established in mature albino rats in some instances by finding the sperms in a vaginal smear, in others by abdominal palpation. They were then segregated in individual cages and the date of parturition recorded. The lactating mothers nursed the litters continually through the experimental period. At regular intervals during the lactation period from the fourth to the twenty-second day using ether anesthesia, a single horn of the uterus was perforated with a needle in such a manner as to insure multiple irritations to the endometrium at several levels. From 3 to 8 days following the operative stimulation of the endometrium, anestrus was determined by vaginal smear. The animals were then killed and the entire reproductive tract fixed in Bouin's fluid. Microscopic examination was used to determine the presence of deciduoma in all instances.

---

1 Lyon, R. A., and Allen, W. M., *Am. J. Physiol.*, 1938, **122**, 624.
2 Weichert, C. K., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 490.
3 Allen, W. M., *Anat. Rec.*, 1931, **48**, 65.
4 Long, J. A., and Evans, H. M., *Mem. Univ. Calif.*, 1922.

TABLE I.
Duration of Deciduomata Initiated During Lactation.

| No. of Rats | (Postpartum) Day Stimulated | (Postpartum) Day Killed | Survival Time of Deciduomata (Days) |
|---|---|---|---|
| 6 | 5 | 8-16 | 9 |
| 6 | 7 | 10-18 | 9 |
| 5 | 9 | 12-20 | 9 |
| 5 | 11 | 14-20 | 8 |
| 4 | 13 | 16-22 | 7 |
| 5 | 15 | 18-22 | 5 |
| 5 | 17 | 21-23 | 4 |

*Results.* The period during which deciduomata may be produced by endometrial stimulation in the lactating rat extends from the fifth to the seventeenth postpartum day. The survival periods of the deciduomata, produced at graded intervals, are presented in Table I. In the initial half of lactation, these growths persist for about 9 days. During the latter part of the nursing period they involute sooner. Those produced at the seventeenth day start to involute almost as soon as they are formed.

It is interesting that toward the end of the lactation period, conditions are favorable for the initiation but not for the maintenance of the growths. Thus by the thirteenth day a fifth-day stimulated deciduoma will have involuted whereas the new thirteenth-day stimulation will be productive of a typical growth.

Early lactation period deciduomata disappeared prior to the occurrence of the first estrus by several days. However, the deciduomas produced later in lactation may disappear as a part of the preestrual changes. These considerations suggest that the estrogen level is so low that the growths do not obtain enough for continued growth. They are notably smaller than the corresponding pregnancy deciduomas during the presence of which enough estrogen is present to present mucification of the vaginal mucosa. The fact that they can be produced suggests that the minimal quantity of estrogen known to be present is adequate for the production of deciduomas of limited size.

*Conclusions.* Deciduomata have a limited survival period during lactation in the rat. Their duration tends to be shorter when produced after the eleventh postpartum day.

10339 P

# Electro-Encephalographic Localization of Atrophy in the Cerebral Cortex of Man.*

MORTON A. RUBIN.

*From the Biological Laboratories, Clark University, and the Research Service, Worcester State Hospital, Worcester, Mass.*

Atrophy of the cerebral cortex involving the fronto-parietal-occipital areas to a greater or lesser degree has been demonstrated in many schizophrenic patients.[1] From our present knowledge of electro-encephalography, one would expect to find some alteration in the relation between the electrical activity of an atrophied region and that of adjacent, normal tissue.

The relationship between the major architectonic regions of the cerebral cortex in "normal" man has been described in terms of their alpha (10 per second) activities.[2] The method consists in determining the amount of alpha wave activity *(per cent time alpha)* in both hemispheres simultaneously from electrodes placed in 7 standard positions along the head about 3 cm to either side of the midline. The electrodes are placed on the scalp over the following cortical regions: tip of the occipital lobe; occipito-parietal fissure; mid-parietal; central fissure (fissure of Rolando); the last 3 electrodes divided the frontal lobe into 3 approximately equal parts. Curves of the distribution of *per cent time alpha* over the 2 hemispheres of "normal" subjects run parallel to each other.

In the present study, 2 to 4 electro-encephalograms were recorded from each of 14 patients, and distribution curves plotted. The patients were selected by Dr. A. Angyal on the basis of certain psychotic symptoms[3] which they exhibited. Very few patients showed neurological signs of cortical pathology.

Records from 6 patients showed one or more cortical regions in which the *per cent time alpha* increased in one hemisphere and decreased in the other, although the electrical activity of both hemispheres ran parallel in the adjacent regions. This was taken as a sign of atrophy. One patient gave a doubtful picture, and 7 were negative. It was not possible to obtain pneumo-encephalograms on all 14 pa-

* Aided by a grant from the Child Neurology Research (Friedsam Foundation).

1 Bateman, J. F., *Arch. Neurol. and Psychiat.*, 1925, **14**, 616; Jacobi, W., and Winkler, H., *Arch. f. Psychiat. u. Nervenkr.*, 1927, **81**, 299; Moore, M. T., Nathan, D., Elliott, A. R., and Laubach, C., *Am. J. Psychiat.*, 1933, **12**, 801.

2 Rubin, M. A., *J. Neurophysiol.*, 1938, **1**, 313.

3 Angyal, A., *Arch. Neurol. and Psychiat.*, 1936, **35**, 1029.

TABLE I.
Comparison of Electro-encephalographic and Pneumo-encephalographic Findings in 9 Schizophrenic Patients.

| Patient | Electro-encephalogram | Pneumo-encephalogram |
|---|---|---|
| H.A. | ++ | ++ |
| C.B. | + | + |
| I.B. | — | + |
| A.B. | + | ++ |
| M.C. | — | — |
| J.D. | — | — |
| E.D. | + | + |
| G.H. | ++ | ++ |
| B.M. | — | — |

\+ Limited to small area (usually parietal or posterior part of frontal).
++ Extensive.
— No atrophy.

tients, but a sufficient number were secured to allow comparison with the electro-encephalographic localizations (Table I). In all cases the electro-encephalographic diagnoses were made before the pneumo-encephalograms were taken.

It is interesting that slow, "pathological" potentials, usually associated was brain lesions,[4] were observed only in the 2 patients who had the most marked cortical atrophy.

*Summary.* It was found possible to localize atrophy of the cerebral cortex in schizophrenic patients by comparing the alpha (10 per second) rhythms of the various regions of the two hemispheres with each other. The findings were confirmed by pneumo-encephalography in 8 out of 9 patients.

10340

## Simplified Rations for Guinea Pigs Suitable for Assay of the Grass Juice Factor.*

G. O. KOHLER, S. B. RANDLE, C. A. ELVEHJEM AND E. B. HART.

*From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.*

Previous publications from this laboratory have described studies

[4] Walter, W. G., *Proc. Roy. Soc. Med.*, 1937, **30**, 579; Williams, D., and Gibbs, F. A., *N. E. J. Med.*, 1938, **218**, 998.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the Research Fund of the University, and the American Butter Company, Kansas City, Missouri.

on the grass juice factor in which both rats[1, 2] and guinea pigs[3] have been used for assay purposes. The basal ration used for each of the species was mineralized winter milk. Since certain laboratories have difficulty in obtaining milk from cows fed rations known to be deficient in the grass juice factor, we were interested in developing a purified ration which could be used in place of the milk. This paper gives a preliminary account of the results obtained when dry rations designed to be low in the grass juice factor were used for guinea pigs.

The following rations were used:

| | P-1 | P-2 | P-3 | P-4 | P-5 |
|---|---|---|---|---|---|
| Dextrin | 70 | 69 | 59 | 59 | 59 |
| Casein (Labco) | 18 | 18 | 18 | — | — |
| Casein (crude) | — | — | — | 18 | 18 |
| Salts 1 | 4 | 4 | 4 | 4 | 4 |
| Brewer's yeast (Pabst) | 4 | 6 | 6 | 6 | 6 |
| 92% alcohol soluble liver extract | 1 | — | — | — | — |
| Cottonseed oil | 2 | 2 | 2 | 2 | — |
| Cod liver oil | 1 | 1 | 1 | 1 | 1 |
| Snowdrift | — | — | 5 | 5 | — |
| Butter fat | — | — | 5 | 5 | — |
| Lard | — | — | — | — | 12 |

The diet and water were supplied *ad libitum*. Each guinea pig was given 2 cc of orange juice daily to supply the vitamin C requirement. The above diets invariably maintain rats in good condition although these animals may show lower than normal rates of growth depending upon previous history. However, when guinea pigs weighing 300-450 g were placed on any one of these rations, death occurred in 3 to 10 weeks. Among the symptoms observed prior to death were severe loss in body weight, muscular atony and weakness, thinning of the hair coat, and stiffening of the joints. In some cases head retraction was noted immediately preceding death. Autopsy showed no signs of rickets or scurvy. In some cases darkened areas on the lungs were observed, suggesting that the animals in their enfeebled condition had contracted pulmonary pneumonia which probably was the terminal cause of death. In many of the animals, however, the stomach linings were found to contain numerous petechial hemorrhages, accompanied in most cases by hemorrhagic ulcers. In some cases these hemorrhages were apparently severe enough to cause death, and in such animals no evidences of pneumonia could be seen.

1 Kohler, G. O., Elvehjem, C. A., and Hart, E. B., *Science*, 1936, **83**, 445.
2 Kohler, G. O., Elvehjem, C. A., and Hart, E. B., *J. Nutr.*, 1937, **14**, 131.
3 Kohler, G. O., Elvehjem, C. A., and Hart, E. B., *J. Nutr.*, 1938, **15**, 445

The addition of dried grass† to the ration P-1 at a level of 5% had little or no beneficial effect on any of the symptoms described. Ten percent of the grass produced a temporary increase in weight, but after about 3 or 4 weeks the animals stopped growing, began to lose weight, and finally died. When 20% of grass was fed, still greater improvement resulted. Extremely variable growth was obtained in guinea pigs receiving rations P-1, P-2, and P-3. However, those receiving ration P-4 or P-5 supplemented with 20% of grass grew at a rate of 4.5 to 5.5 g per day over a period of 8 weeks. Autoclaving the grass for one hour at 15 pounds pressure completely destroyed the growth-promoting activity.

In several cases animals receiving 20% of unautoclaved grass suddenly sickened during rapid growth, and died after losing much weight during the following 2 or 3 days. Autopsy showed severe stomach ulcers. Further, one of the animals, which had grown to maturity at a normal rate while receiving ration P-4 plus 20% of grass, began to lose weight rapidly after 15 weeks on this ration. He finally died with severe stomach ulcers. These observations, together with the fact that guinea pigs receiving a milk diet deficient in the grass juice factor show no stomach ulcers, suggest that the basal dry rations used are deficient in at least 2 nutritional factors, namely, the grass juice factor and a factor associated with the maintenance of a normal stomach lining. Grass supplies plenty of the former, but is low in the latter. Since it has been shown that cholic acid and chondroitin prevent gizzard lesions in the chick,[4, 5] these compounds were fed as supplements to ration P-4 at levels of 0.5 and 3.0% respectively. Severe stomach hemorrhages and ulcers were obtained in guinea pigs receiving these supplements. Hence it must be concluded that the guinea pig stomach ulcer factor is distinct from the chick gizzard factor. In spite of the complicating effect of the stomach ulcers, ration P-5 may be used with considerable success for the assay of the grass juice factor if care is taken to associate sudden losses in weight with the presence of stomach ulcers, which can be verified upon autopsy. A few typical results are shown in Chart 1.

It is evident from these results that 2 nutritional factors may be studied through the use of guinea pigs maintained on purified diets

† We are indebted to Mr. L. H. Smith and Dr. W. R. Graham, Jr., of the Cerophyl Laboratories, Division of American Dairies, Kansas City, for generous supplies of dehydrated cereal grasses (Cerophyl).

4 Bird, H. R., Oleson, J. J., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, in press.

5 Almquist, H. J., and Mecchi, E., *J. Biol. Chem.*, 1938, **126**, 407.

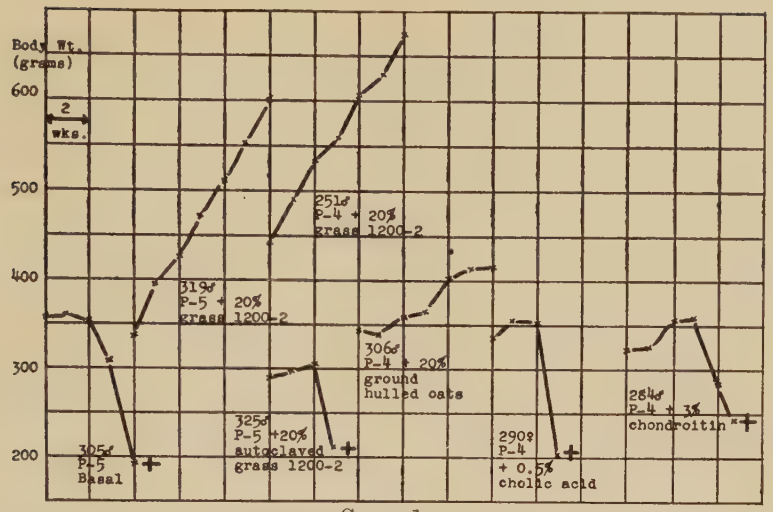



CHART 1.

Growth curves of guinea pigs fed rations P-4 and P-5 plus various supplements. Pigs 284, 290, and 305 showed severe stomach ulcers upon autopsy.

such as P-4 and P-5. The growth-promoting factor is undoubtedly the grass juice factor and is supplied from properly prepared young grass. In this connection it is interesting to refer to a paper by Wulzen and Bahrs[6] which was called to our attention by Dr. Ira Manville. These workers describe a condition in guinea pigs very similar to the deficiency of the grass juice factor which can be prevented by fresh kale or fresh alfalfa.

The second factor, which may be called the stomach ulcer factor, may not be supplied in adequate amounts by 20% of grass. It is apparently present in fair amounts in grains since guinea pigs have been kept on ration P-5 plus 20% ground hulled oats for 15 weeks with no indication of stomach ulcers.

## 10341

## Effect of Gelatine on Muscular Fatigue.

G. B. RAY, J. R. JOHNSON AND M. M. TAYLOR.

*From the Department of Physiology and Pharmacology, Long Island College of Medicine, Brooklyn, N. Y.*

It has been reported by Boothby[1] that the onset of muscular

6 Wulzen, R., and Bahrs, A. M., *Physiol. Zool.*, 1936, **9**, 508.

1 Boothby, W. M., *Proc. Mayo Clin. Staff Meetings*, 1934, **9**, 600.

fatigue can be delayed by the addition of a considerable amount of glycine to the diet of the normal individual. He has further shown that in cases of myasthenia gravis and similar diseases the feeding of glycine tends to restore the wasted muscle tissue, thus indicating an effect upon the physiological state of these tissues. Wilder[2] believed his ability to play tennis increased after 2 weeks on a glycine diet. The possible explanation of these reactions is to be found in the creatinogenic action of glycine and the storage of at least part of this product in the muscles.[3] This is quite in accord with the current concept of the chemistry of muscular contraction wherein creatine in its combination with phosphoric acid plays an important rôle. The concept of storage is confirmed by the work of Rose, Ellis, and Helming,[4] who found a male subject on a creatine diet could store this substance. This was not evident in the case of a female subject.

Unfortunately, Boothby found that normal subjects were unable to take large amounts of glycine without some discomfort.[1] It seemed of interest, therefore, to determine if a food rich in glycine would also increase the amount of work a trained subject was capable of doing before fatigue set in. Among the dietary possibilities, perhaps the most outstanding is gelatine, which contains about 25% glycine, is easily digested, and is readily available as a food substance.

To the authors' knowledge, none of the above papers, concerned with muscular fatigue and glycine, reported quantitative studies on the work output of their subjects. It was felt, therefore, that it was important that the amount of work done be measured as accurately as possible, both during a training period and during a period when gelatine was added to the normal diet. For this purpose a bicycle ergometer of special design was used. The essential part is a small self-exciting 110 volt A. C. generator driven by the rear wheel of a stationary bicycle. The load on this generator depends upon a series of lamps in parallel and the output is read on a watt-meter.* Thus, by observing the output in watts and timing the duration of work, the total energy developed during the exercise period can be calculated.

Ten subjects, 6 men and 4 women, were trained on the ergometer

[2] Wilder, R. M., *Proc. Staff Meetings Mayo Cl.*, 1934, **9**, 608.

[3] Tripoli, C. J., and Beard, H. H., *Southern Med. J.*, 1938, **31**, 662.

[4] Rose, W. C., Ellis, R. H., and Helming, O. C., *J. Biol. Chem.*, 1928, **77**, 171.

* The writers wish to acknowledge with thanks the courtesy of the General Electric Company in supplying the necessary special meter and generator.

until the maximal output of work before fatigue set in was as constant as could be expected under the experimental conditions. After a satisfactory training period, the end of which was determined by the constancy of the daily work, the subjects were placed on a gelatine† diet. Three of the women subjects took 45 g per day, the fourth took 67.5 g. The men were all given 60 g. It is realized that these amounts are, to a degree, arbitrary but do approximate in the case of the men a dose of 15 g of glycine which is within the range used by Adams, *et al.*, for normal subjects.[5] It was

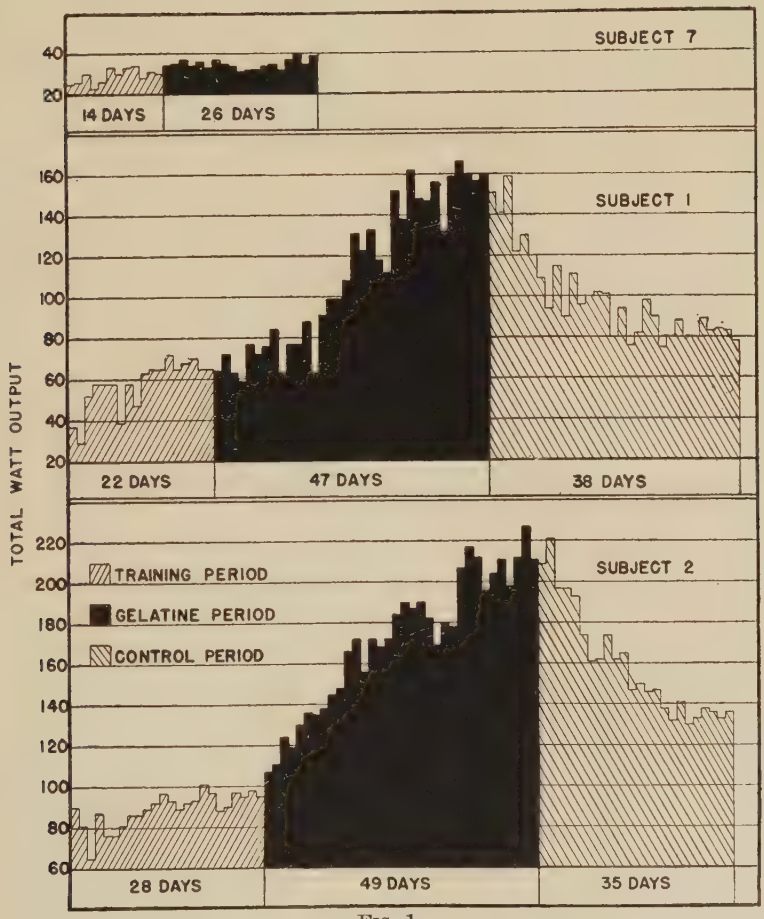



FIG. 1.

† The writers wish to thank the Knox Gelatine Laboratories for their cooperation and the supply of gelatine.

5 Adams, M., Power, M. H., and Boothby, W. M., *Am. J. Physiol.*, 1935, **111**, 596.

found that the most convenient way of administering these large amounts of gelatine was to mix it with orange and lemon juice. If the juice was well chilled, as much as 30 g of gelatine could be given in 8 ounces of juice and the whole taken before the gelatine swelled appreciably. In this manner it was possible for one to take the entire assigned amount of gelatine without distress.

The details of 3 representative experiments are given in Fig. 1. The cross-hatched area to the left indicates the training period; the solid black, the period during which gelatine was administered, and the area to the right, the control or recovery period during which fruit juice alone was given the subjects. It was felt necessary to include this period in order to demonstrate that any effect was due to the gelatine rather than to the increased intake of vitamins.

The record of Subject 7, at the top of the chart, that of a woman medical student, is presented since it is one of 2 records obtained from women subjects showing a semblance of an increase (3%) during the gelatine diet. She received 45 g of gelatine (the equivalent of 11.25 g of glycine) a day during the experimental period. The slight increase noticed is of dubious significance since it may well be assigned to a possible effect of a lengthened training period.

The second graph, that of Subject 1, represents a complete record on a man 25 years old. After the usual period of training, he received 60 g of gelatine a day. The gelatine was continued until the fatigue level appeared to have reached its maximum. In the case of this individual it was at the end of 47 days. At that point the gelatine was discontinued but the 16 ounces of orange and lemon juice maintained. It will be noted that shortly after the cessation of gelatine, the total work output fell off and at the end of 38 days came to a level. At this point the experiment terminated.

Subject 2, whose complete record is also presented, gives a picture similar to that of Subject 1. There is one notable exception in that the response to gelatine is very prompt. This subject, an instructor, aged 33, was of slight build. It is interesting to record at this time that during the gelatine period he gained 10 pounds, 8 of which were retained to the end of the experiment.

In conclusion, it may be said that men, when given adequate amounts of gelatine, are invariably able to increase the amount of work produced before fatigue sets in. The results varied from 37% to 240% increases above the training level. In by far the greater number of subjects the increase was more than double the pregelatine work level. This is not true in the case of women sub-

jects, where no appreciable effect was noticed. It might be supposed that these effects were due to the considerable amounts of orange juice and its vitamins. When, however, gelatine was discontinued and the same amount of fruit juice maintained, the work output rapidly fell to an approximation of the pregelatine level, thereby indicating that the effect was primarily that of the addition of gelatine to the diet. The sexual variation is also of interest from the point of view of the intepretation of the results. As mentioned above, Rose, *et al.*, found a marked sexual variation in the ability to store creatine. These experiments are most suggestive since they indicate a situation comparable to that found in our own experiments. It would seem as though this action of gelatine were particularly concerned with the creatinogenic action of its amino acids, especially glycine.

**10342**

## Effect of the Experimental Production of an Accessory Blood Supply Upon Normal Kidney.

HARRY A. DAVIS AND I. F. TULLIS, JR. (Introduced by P. W. Smith.)

*From the Department of Pathology, University of Tennessee.*

The present report is concerned with the effects of the experimental production of an accessory blood supply upon the structure and function of the normal kidney.

Eleven dogs, weighing from 6 to 15 kilos, were used. In 7 of these dogs an accessory circulation was produced in the left kidney and, in 4, in the right kidney. Under general ether anesthesia a mid-line incision was made into the peritoneal cavity. The capsule of the kidney to be used was removed. A small protected arterial clamp was placed upon the renal artery. An incision was made in the antihilar surface of the kidney extending longitudinally from the upper to the lower pole. The incision extended in depth to, but not through, the renal pelvis. Little bleeding occurred from the kidney during this phase of the operation. The omentum was lifted up from the peritoneal cavity and gently packed into the gaping incision in the renal parenchyma. Four sutures of No. 0 chromic catgut were passed through the renal tissue and omentum, which was thus anchored to the kidney. Following the release of the arterial clamp, a moderate amount of bleeding occurred, but this soon ceased.

The abdominal wound was closed in the usual manner. Two of the dogs were subsequently reoperated upon, and the renal arteries of the involved kidneys were divided between ligatures. After a period of 10 days to 6 weeks from the primary operation, the functional value of the accessory renal circulation was examined. There was no operative mortality in this series of animals. The animals recovered quickly from the operative procedure.

The histological changes in operated kidneys are of interest. The omentum quickly becomes adherent to the renal parenchyma. The fatty connective tissue of the omentum in the depths of the renal incision becomes partially replaced by fibrous connective tissue. Large arterial and venous blood channels can be found running from the omentum into the renal parenchyma, and branching out into many fine capillaries. The vascularization of the entire operated kidney is considerably greater than that of the non-operated kidney. The intertubular and glomerular capillaries of the artificially vascularized kidney are distended. After a variable period of time, the operated kidney shows some diminution in size, and, microscopic evidence appears of a relative increase in the number of glomeruli and a marked dilatation of the renal tubules. A moderate number of phagocytic monocytes containing haemosiderin pigment was present in the fibrous connective tissue. Microscopic study of the unoperated kidneys revealed no pathology.

In Table I are presented representative data concerning the water diuresis in the vascularized kidney compared with the normal kidney upon the opposite side. The ureter on each side was cannulated under nembutal anesthesia, and the character and volume of the urine from each kidney was determined following the intravenous injection of 1000 cc of 5% glucose solution or of 0.85% sodium chloride solution. For a period of 10 days after operation, the urine from the operated kidney is of low specific gravity and contains red blood cells and albumin. Thereafter, the specific gravity rises and the urine becomes free from albumin and red blood cells. The specific gravity of the urine from the operated kidney, however, tends to remain slightly lower than that from the normal kidney. Water diuresis was greater—5% to 100% increase—from the artificially vascularized kidney in 8 out of 9 animals, which were examined from this standpoint. Preliminary studies of renal function were carried out, using the urea clearance and P.S.P. tests. The results in one animal are shown in Table II, and would suggest that the experimental production of an accessory circulation to a kidney, by the method described, enhances its work output. The operated kidney, in the data presented, was capable of clearing 28.3 cc of

TABLE I.
Experimental Production of Accessory Blood-supply to a Kidney upon Its Urine Secretion Function.

| Dog Wt Kg | Type of injection | | 1 hr | 2 hr | 3 hr | 4 hr |
|---|---|---|---|---|---|---|
| | | | Urine Secretion, cc | | | |
| Exp. 5 | 5% Glucose Sol. | *Left kidney | 34 | 44 | 51 | 86 |
| 10 | | Right " | 10 | 16 | 18 | 50 |
| Exp. 6 | 5% Glucose Sol. | *Left " | 26 | 44 | 50 | 56 |
| 12 | | Right " | 8 | 15 | 16 | 18 |
| Exp. 11 | 5% Glucose Sol. | Left " | 60 | 114 | 142 | 143 |
| 6 | | *Right " | 64 | 120 | 162 | 177 |
| Exp. 10 | 0.85% NaCl Sol. | Left " | 0 | 4 | 12 | 34 |
| 11 | | *Right " | 5 | 12 | 24 | 48 |

*Provided with accessory blood supply.

TABLE II.
Influence of an Accessory Blood Circulation upon Renal Function.

| Exp. 12 A | | | | | | | Exp. 12 B |
|---|---|---|---|---|---|---|---|
| Dog wt 8.5 kg | Total quantity urine, cc | Sp. Gr. | pH | Urine urea nitrogen, mg % | Blood urea nitrogen, mg % | Urea clearance test (Cs) | P. S. P. Test % of dye excreted |
| *Right kidney | | | | | | | |
| 1st hr | 88.5 | 1015 | 7.1 | 61.6 | 9.32 | 8 cc | (Urine secretion = 178 cc) 40% |
| 2nd " | 76.5 | 1022 | 7.1 | 168.0 | — | 20.3 " | (Urine secretion = 27 cc) 5% |
| Left kidney | | | | | | | |
| 1st hr | 83.5 | 1020 | 7.1 | 72.8 | — | 9.2 " | (Urine secretion = 84 cc) 25% |
| 2nd " | 51.5 | 1023 | 7.1 | 44.8 | — | 4.42 " | (Urine secretion = 3 cc) 2.5% |

*Supplied with accessory blood circulation.

blood of its urea content per minute in comparison with the 13.62 cc cleared by the opposite normal kidney. Over a period of 2 hours, the operated kidney excreted 45% of the injected phenolsulphonephthalein compared with the excretion of 27.5% from the opposite normal kidney. The intravenous injection of 10 cc of 20% urea solution produced a greater diuresis from the kidney with the accessory circulation than from the unoperated kidney.

The data here presented suggest that the experimental production of an accessory blood supply to the kidney is practicable. Such an additional blood circulation may have a functional value, inasmuch as diuresis, urea clearance, and phenolsulphonephthalein excretion are somewhat greater from a kidney provided with such an extra blood supply, as indicated by preliminary renal function tests. Further evidence of the establishment of an additional blood circulation by the outlined operation is provided by the fact that the injection of 10 cc of a 1% solution of methylene blue into the femoral vein produces a diffuse staining of the renal parenchyma after ligation and division of the renal artery, provided that an accessory blood supply is maintained to the kidney through the omentum.

*Conclusion.* An operation is described for the production of an accessory blood supply to the kidney by the way of the omentum. The effects of the procedure in 11 dogs are described.

**10343**

## Concentration of Sulfanilamide in Spinal Fluid and Blood Following Single Intrathecal Injection of Drug.

E. NETER, D. H. WEINTRAUB AND A. L. DAYMAN.

*From the Laboratories and the Medical Service of the Children's Hospital, Buffalo, New York.*

Sulfanilamide, which is successfully used in the treatment of patients suffering from purulent meningitis due to beta-hemolytic streptococcus and meningococcus, is readily absorbed after oral administration and is found in the blood and spinal fluid; usually, the concentration in the spinal fluid is somewhat lower than in the blood (Marshall, Emerson, and Cutting[1, 2]). Some authors, including

[1] Marshall, E. K., Emerson, K., and Cutting, W. C., *J. Am. Med. Assn.*, 1937, **108**, 953.

[2] Marshall, E. K., Emerson, K., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 196.

Schwentker, Gelman and Long[3] as well as Crawford and Fleming[4] recommend that sulfanilamide may be given in certain cases by intrathecal injection. This form of administration is indicated particularly when treatment by other routes is not successful. It seemed desirable to determine what concentrations of sulfanilamide are reached and maintained in spinal fluid and in blood following intrathecal injection of the drug. In the following note, observations are reported dealing with this problem.

The studies were carried out in the following manner: Patients on whom a lumbar puncture was indicated for diagnostic reasons and whose spinal fluid was found to be normal, were given intrathecally 10 cc of an 0.8% solution of sulfanilamide (Prontylin, repurified for injection, Winthrop) in physiological saline solution. Four and 18 hours following this injection, lumbar punctures again were performed, and at the same time blood specimens were taken. The concentration of sulfanilamide in both spinal fluid and blood, was determined according to the method described by Marshall, Emerson and Cutting[1]; 2 standard solutions, containing 10 mg % and 1 mg % of sulfanilamide respectively were used.

TABLE I.
Concentration of Sulfanilamide in Spinal Fluid and Blood Following a Single Intrathecal Injection of 80 mg of Sulfanilamide.

| Hrs following administration of sulfanilamide | | Concentration of Sulfanilamide in mg % in Spinal Fluid | | Concentration of Sulfanilamide in mg % in Blood | |
|---|---|---|---|---|---|
| | | Case I | Case II | Case I | Case II |
| (a) | 4 | 11.2 | 16.2 | 0.7 | trace |
| (b) | 18 | negative | trace | negative | negative |

Negative = no sulfanilamide demonstrable.
Trace = less than 0.1 mg %.

The results obtained in 5 patients were essentially the same. Table I presents the findings of 2 of the cases. It may be seen from this table that 4 hours after the intrathecal injection of 80 mg of sulfanilamide a relatively high concentration (10-16 mg %) of the drug is present in the spinal fluid, but that the concentration in the blood at this time is much lower; in our series, the concentration of sulfanilamide in the blood amounted to 1 mg % or less. Eighteen hours following a single intrathecal injection of 80 mg of sulfanilamide, the concentration in both spinal fluid and blood was very low or the drug could not be demonstrated at all. It is obvious that

3 Schwentker, F. F., Gelman, S., and Long, P. H., *J. Am. Med. Assn.*, 1937, **108**, 1407.

4 Crawford, T., and Fleming, G. B., *Lancet*, 1938, **1**, 987.

variations in the concentration of sulfanilamide in different individuals are to be anticipated, since the resulting concentration of the drug depends not only upon the dosage given but also upon the weight of the individual, the amount of spinal fluid present, the intake of fluids, the efficiency of the kidney function, and so forth. The data presented may possibly serve as an aid in the treatment of purulent meningitis and may point to certain advantages and disadvantages of the intrathecal administration of sulfanilamide. Observations in cases of meningitis concerning the concentration of sulfanilamide in blood and spinal fluid, following a single intrathecal injection were not carried out for obvious reasons, nor was the concentration of sulfanilamide in cerebro-spinal fluid obtained by cisternal puncture determined. It is possible, as Dr. E. K. Marshall, Jr.,[5] suggested that the concentration of the drug is lower in the cisternal fluid than in spinal fluid.

From the observations presented above it may be concluded that: (1) It is possible to obtain a relatively high concentration (10-16 mg %) of sulfanilamide in the spinal fluid 4 hours after a single intrathecal injection of 80 mg of sulfanilamide to patients free of meningitis; (2) The drug is readily eliminated from the spinal fluid of these patients within 18 to 24 hours. (3) The concentration of sulfanilamide in the blood at the periods of observation is relatively low (1 mg % or less).

**10344**

## Comparative Nutritive Value of Firm and Watery Egg Albumen.*

N. B. GUERRANT AND W. J. RUDY.

*From the Pennsylvania State College.*

The question of egg quality has become a matter of economic concern to the producer, the distributor and to the consumer. Investigators who have attempted to study this problem have been unable to agree as to what constitutes egg quality. Needless to say this lack of agreement has been a considerable handicap to researches along this line. However, in spite of this lack of agreement as to the characteristics of quality, eggs with excessive amounts of watery

[5] Marshall, E. K., Jr., personal communication.

*Authorized for publication on November 19, 1938, as paper No. 861 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

white have been discriminated against by the consumer and also by the wholesale distributor, mainly because the former has found that such eggs present a less desirable appearance when poached or fried than do eggs having the firm white. In contrast to this particular objection, some researches have shown that the watery egg white has more desirable whipping qualities than does the firm white. While watery and firm albumen differ markedly in physical characteristics, no data are available to show that they differ in nutritive properties.

Because of the increasing interest in the subject of egg quality, it appeared to us that an effort should be made to determine whether the physical state of the albumen (watery or firm) of a freshly laid egg is an index of nutritive value. With this point in view, a series of studies were made to determine the nutritive value of the two types of albumen as indicated by their relative capacity to produce growth in healthy young rats.

The eggs used in these studies were obtained from hens that were receiving an all-mash ration. The eggs were gathered and their fractionation carried out on the same day that they were laid. The method employed in fractionating the eggs was as follows: After breaking the shell, the total albumen was carefully separated from the yolk and poured on a brass sieve (openings 2.14 mm in diameter) while the yolk itself was placed in a separate glass receptacle. That portion of the albumen which passed through screen was designated as the watery albumen while that retained by the screen was likewise designated as firm albumen.

After all eggs had been fractionated in this manner, each of the 3 portions, *viz.*, yolks, watery albumens, and firm albumens, was thoroughly mixed and weighed in order to determine its relative percentage in the original eggs. A weighed portion of the yolk and of the watery albumen was recombined so as to give a mixture having the same relative composition as the original eggs. A similar mixture was made using weighed portions of the yolk and firm albumen. Each mixture of yolk and albumen was then coagulated by heating in a double boiler, broken into small lumps and dried on cheesecloth in a current of warm air. The dried product was mixed, ground to a satisfactory degree of fineness and stored in an electric refrigerator until fed to the test animals. For the first nutritional test the eggs were prepared in small weekly batches, while for the second test sufficient eggs were prepared at the beginning of the experiment to last during the entire period.

The nutritional studies consisted in feeding carefully matched groups of healthy young rats, maintained in individual metal cages, a diet consisting of one of the egg combinations. In order to bring

about a more favorable mineral balance calcium carbonate was fed *ad lib.* It was believed that differences in the nutritive value of the 2 types of albumen would be more readily demonstrated if the test diet consisted entirely of the egg products. The amount of egg fed daily was restricted so that each test animal consumed the same amount of the dried egg during the weekly period. The amount of

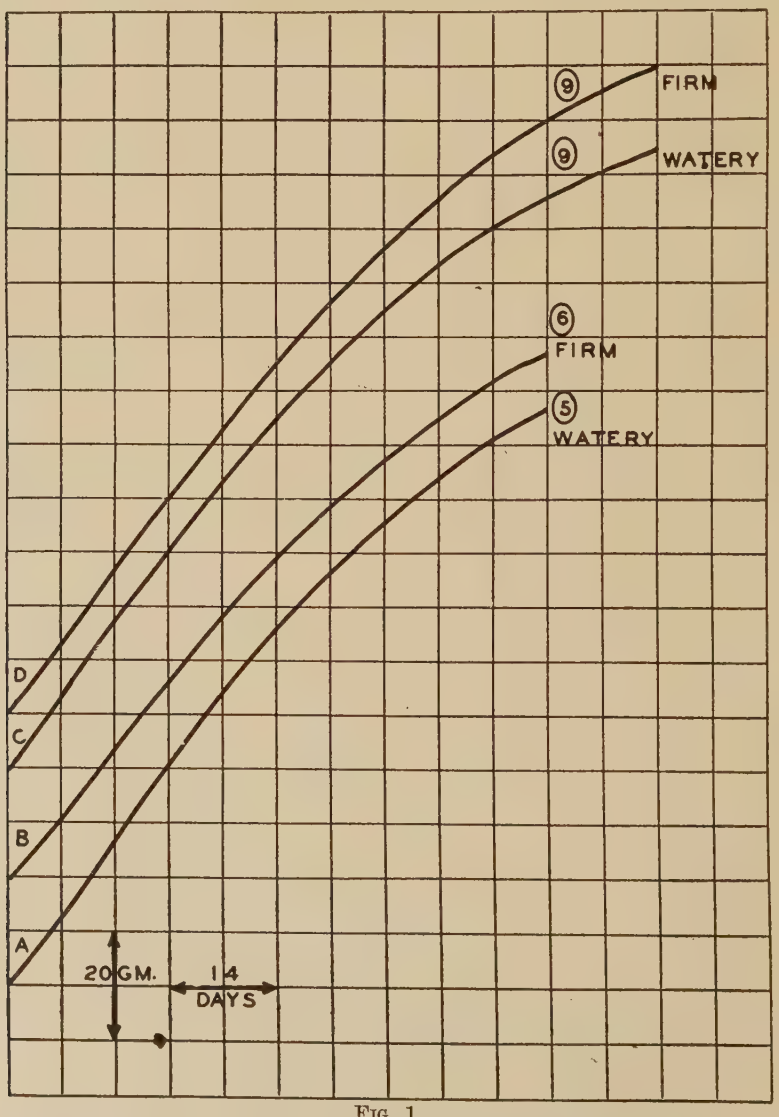



FIG. 1.

Average growth responses of young rats receiving diets composed entirely of dried eggs. These diets differd only in the type of albumen which they contained.

diet was increased from week to week in order to compensate for increases in body weight of the animals. A liberal amount of calcium carbonate (U.S.P. grade) was maintained in a separate container in each cage. Each animal was weighed at weekly intervals and was given the usual care and attention accorded experimental animals. The first feeding test was continued for a period of 10 weeks while the second test lasted 12 weeks. It was believed that experiments of this duration were sufficient to determine whether or not marked differences existed in the nutritive value of the two types of albumen.

The growth responses made by these groups of animals are presented in the accompanying graph.

Curves A and B represent the average growth response of the test animals used in the first feeding test, while curves C and D represent similar data for the animals used in the second test. The enclosed numerals denote the number of test animals considered in each feeding test.

It may be observed from the above graph that the growth responses made by the animals receiving the diets containing the 2 types of albumen are quite similar for both feeding trials. In the first test (Curves A and B) the animals which received the watery albumen made slightly greater gains in body weight than did their litter mates which received a similar diet containing firm albumen. The growth response made by the test animals used in the second feeding test (Curves C and D) was of approximately the same magnitude as that of the first test, but in this trial the animals receiving the diet containing the firm albumen made the greatest average gain. The average gains for the 12-week period were 119 and 115 g, respectively.

When the above growth responses were considered as a whole, from the standpoint of growth production per unit weight of moisture-free diet consumed, it was found that one gram of the diet containing the firm albumen produced an average gain in bodyweight of 0.2817 g, while an equal weight of the diet containing the watery albumen produced a gain of 0.2813 g. Such differences in growth responses are not significant. It, therefore, appears from the data obtained in the above feeding tests that there is no difference in the nutritive value of the 2 types of egg albumen.

*Summary.* Groups of young rats were fed diets composed solely of dried egg products, the difference in the diets being the type of albumen which they contained. The resultant growth of these animals indicates that there is no difference in nutritive value of watery and firm albumen.

## 10345 P

## Clinical Experience with Globin Insulin.

LOUIS BAUMAN.

*From the Departments of Surgery and Medicine, Presbyterian Hospital and Columbia University, N. Y.*

A clinical trial of globin insulin[1] was made on approximately 25 patients suffering from diabetes of varying degrees of intensity. The preparation was given in a single dose, usually 30-45 minutes before breakfast. Whenever possible, its action was compared with standard and protamine zinc insulin. The sugar in the urine was usually determined in 2-hour periods. Four finger blood sugars taken before breakfast, lunch, dinner and 10 P.M. respectively were also recorded at frequent intervals. The maximum single dose was 145 units. Skin reactions were never encountered. We were using a water-clear solution. When hypoglycemic reactions occurred, the time was usually in the late afternoon, whereas protamine zinc in-

TABLE I.

H.D. Age 42. History No. 354588. Diabetes discovered in 1933.
Data of Previous Admissions.

| Dates | Diet, Calories | | | Insulin Units | | | Regulation |
|---|---|---|---|---|---|---|---|
| | Carb. | Prot. | Fat | A.M. | M | P.M. | |
| 9/17/33-9/27/33 | 100 | 60 | 50 | 30 "S" | 0 | 18 "S" | Excellent |
| 4/30/37-5/13/37 | 250 | 75 | 100 | 44 "S" | 0 | 22 "S" | Good |
| 5/17/37-5/17/37 | 250 | 75 | 100 | 40 "S" | 0 | 40 "S" | " |

| Dates | Insulin Units "G" | Diet C. | P. | F. | Blood Sugars Before Breakfast | Lunch | Dinner | 10 P.M. | Urine Sugars 11 A.M. | 4 P.M. | 7 A.M. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1937 | | 250 | 100 | 75 | | | | | | | |
| 12/3 | 70 | | | | | | | | | | |
| 12/7 | 70 | | | | 149 | 309 | 233 | 229 | 0 | ± | ± |
| 12/8 | 70 | | | | 148 | 322 | 204 | 274 | 0 | 0 | + |
| 12/10 | 85 | | | | | | | | 0 | 0 | 0 |
| 12/11 | 85 | | | | 104 | | | | 0 | 0 | 0 |
| 12/13 | 85 | | | | 71 | | | | 0 | 0 | 0 |
| 12/14 | 82 | | | | | | | | 0 | 0 | 0 |
| 12/15 | 82 | | | | 67 | 145 | 118 | 96 | 0 | 0 | 0 |
| 12/16 | 82 | | | | 110 | 172 | 106 | 95 | | | |

Note: Since discharge about 11 months ago she has visited the clinic about 21 times. Regulation has been very satisfactory on a single dose of globin insulin given one-half hour before breakfast.

"S" = Standard Insulin.

"G" = Globin Insulin.

[1] Reiner, L., Searle, D. S., and Lang, E. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 171.

sulin shocks usually occur in the early morning hours. It was found that in practically every case a single dose of globin insulin would regulate the moderately severe and mild cases. In several severe cases, uncontrollable with protamine insulin, fair regulation was obtained with the globin compound. An illustrative table is included.

Thanks are due Drs. Edgar Stillman, Bertram J. Sanger, Henry E. Marks and Rudolph Scharf for their coöperation.

**10346 P**

## Insulin Preparations with Prolonged Activity. I. Globin Insulin.

L. REINER, D. S. SEARLE AND E. H. LANG.

*From the Burroughs Wellcome & Co. U. S. A. Experimental Research Laboratories, Tuckahoe, New York.*

It is believed that an insulin preparation with prolonged activity is usually in the form of a suspension insoluble at the pH of the blood. When insulin was mixed with globin, the solution remained clear at about pH 4 or less, and precipitated between pH 5 and 8. Both the clear and precipitated globin insulin combinations produced a prolonged hypoglycemia when injected into normal animals. The duration of this hypoglycemia is longer in dogs and humans than in rabbits. When 0.2-0.3 mg of zinc was added to a preparation containing 3.8 mg of globin per 100 units of insulin, the duration of the hypoglycemia was more than twice as long as that produced by standard insulin containing the same amount of zinc. As a rule the presence of zinc in the globin mixture intensified and prolonged the hypoglycemia. It was found that the most convenient way to test insulin preparations was to inject into fasting rabbits an amount that failed to produce convulsions in at least 90% of the animals. For comparison the same dose of standard insulin was injected in 2 portions 5 hours apart. The duration of hypoglycemia after injection of globin insulin was not as long as that produced by protamine zinc insulin, but the development of hypoglycemia was very much faster. The characteristic blood sugar curves for rabbits indicate that the hypoglycemia is most intense a few hours after injection and that it disappears gradually.

10347

## Gonadotropic Action of Anterior Pituitary Extract after Tryptic Digestion.

GRAHAM CHEN AND H. B. VAN DYKE.

*From the Department of Pharmacology, Peiping Union Medical College, and the Squibb Institute for Medical Research, New Brunswick, N. J.*

Work which we have had under way during the past 2 years is of interest in connection with the recently published observations of McShan and Meyer.[1] These authors concluded that trypsin largely destroys the luteinizing action of anterior pituitary gonadotropic extract. All our results are in accord with this statement and are based upon experiments requiring 492 normal immature rats and 246 hypophysectomized immature rats.

Three different commercial preparations of trypsin were used. Most of the experiments were performed with a preparation made by Merck (Darmstadt) which has since been found to hydrolyze casein at approximately the same rate as crystalline chymotrypsin made by the method of Kunitz and Northrop. Two lots of trypsin prepared by Grübler also were used; the manufacturer stated that one lot contained 50 Fuld-Gross units per gram, the other 1200 Fuld-Gross units per gram. Digestion usually was carried out in a 0.2% solution of $Na_2CO_3$ over a period of 15 hours at 38°C. At the end of digestion the pH of the solution was about 9.8. All control extracts were treated exactly the same way except that trypsin was not added. Extracts of acetone-desiccated sheep or horse pituitary,* which had been powdered, were ordinarily made by the method of van Dyke and Wallen-Lawrence[2] and represented, roughly, 3% of the weight of crude pituitary powder. Removal of nearly all the luteinizing action of such extracts could be accomplished by digestion of a 0.2-0.3% solution of the extract in 0.2% $Na_2CO_3$ containing 0.1% of Merck's trypsin. Similar results were obtained by employing the weak and strong trypsins of Grübler in concentrations of 0.4 and 0.03% respectively.

Figure 1 illustrates how the degree of ovarian hypertrophy induced by the injection of digested extract may vary according to

[1] McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1938, **126**, 361.

* We are indebted to Dr. A. S. Parkes whose cooperation made possible the purchase of the powdered horse pituitary.

[2] van Dyke, H. B., and Wallen-Lawrence, Z., *J. Pharm. Exp. Therap.*, 1933, **47**, 163.

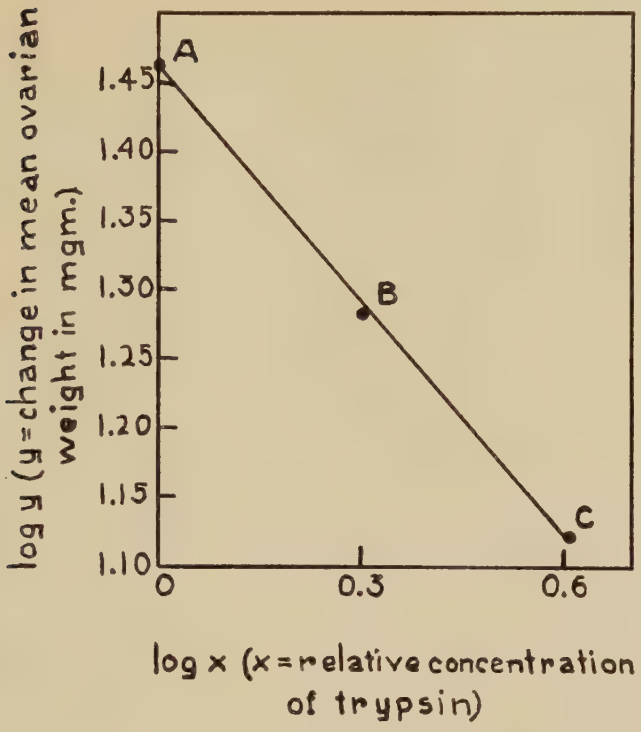



FIG. 1.

The effect of various concentrations of commercial trypsins on the potency of anterior pituitary extract as measured by the change in ovarian weight compared with non-injected controls. Each of the 3 dots represents 17 normal immature rats. All the animals compared were littermates. The same sheep anterior pituitary extract was always used. However, 3 samples of trypsin were employed.

The significance of the differences between the effects of the 3 concentrations of trypsins was calculated by the well known method of Fisher with the following results:

| Groups compared | P |
|---|---|
| A and B | $<0.02$ |
| A and C | $<0.001$ |
| B and C | 0.05 |

the concentration of trypsin used: the change in ovarian weight (hypertrophy) is greater if low concentrations of trypsin are used.† In this group of experiments a 0.2% solution of extract of sheep pituitary powder digested by trypsin in different concentrations was injected into normal immature rats. Although an aliquot of the alkaline solution of the extract incubated under the same conditions caused typical extensive luteinization without apparent loss of po-

† If a crystalline proteolytic pancreatic enzyme had been employed, presumably only the rate of hydrolysis would have been affected by varying the concentration of enzyme. The interpretation of the effect of varying the concentration of the crude preparations of trypsin used may be more complex.

tency, the digested extracts, which alone are indicated in the chart, brought about marked follicular growth associated with some luteinization, especially when low concentrations of trypsin were used. Experiments were therefore undertaken in hypophysectomized rats to determine whether digested extracts are completely free from luteinizing properties.

Both digested and control non-digested but incubated extracts of horse and sheep pituitary extract were injected into hypophysectomized immature female rats. We have not been able to convince ourselves that extracts can be completely freed from "luteinizing hormone" by means of tryptic digestion. Although a starting material such as horse-pituitary extract may be highly potent and produce chiefly a follicular stimulation, yet a large enough dose of this extract after digestion undoubtedly produces the formation of lutein tissue (Fig. 2). Smaller doses of extract may cause definite or marked follicular stimulation without detectable luteinization. In hypophysectomized immature male rats receiving injections for 3-4 days, digested extracts which were highly effective in female rats frequently caused doubling or trebling of the testicular weight (compared with that of non-injected hypophysectomized controls). However, the microscopic changes often were slight. Some tubules of a testis might be well maintained whereas neighboring tubules might appear as atrophic as if no treatment had been given. The number of mitoses in the germinal epithelium sometimes appeared to be increased. Usually tubular diameter was increased; the tubules themselves seemed to be separated by an unusual accumulation of non-cellular fluid. The interstitial cells were completely atrophic; the seminal vesicles were not enlarged.

Neither the crude nor the digested extracts of horse pituitary had any effect on the adrenals or thyroid of hypophysectomized rats receiving doses 20 times that capable of definitely stimulating the ovary.

It was considered possible that "antihormone" might not be produced by digested gonadotropic extract. Accordingly, littermate male and female normal rats, 21-24 days old and weighing about 50-60 g initially, were divided into 3 groups consisting of a non-injected control group, a group receiving non-digested horse pituitary extract, and a group receiving the same extract after digestion. There were 4-6 animals in each of the 6 groups. The injected rats received 0.05 mg of either extract daily at first; the dose was gradually increased until 0.4 mg was administered daily. The period of injection was 46 days. At the end of the experiment the mean weight of the ovaries of the injected females was less than that of

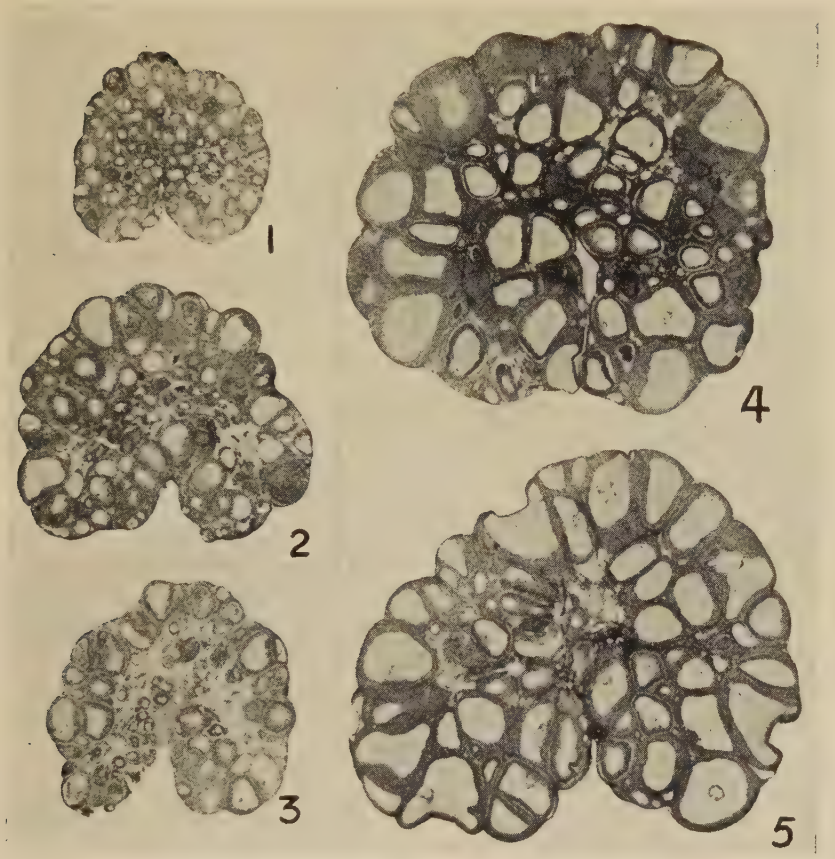



FIG. 2.

Photomicrographs of the ovaries of littermate rats (all × 17 before reduction). All except No. 2, which served as a normal control, were hypophysectomized at an age of 21 days. One-sixth of the dose of extract was given twice daily during the 24-26 days; the animals were killed on the 27th day. The dose of digested extract represented roughly 5 times the quantity of horse-pituitary extract without digestion. No lutein tissue can be found in 3. Lutein tissue is present in 5 but is much less extensive than in 4.

TABLE I.

| No. | Treatment | Weight, in mg, of | | |
|---|---|---|---|---|
| | | Ovaries | Empty uterus | Adrenals* |
| 1 | None | 7.46 | 20.2 | 5.46 |
| 2 | ,, | 18.14 | 52.3 | 15.62 |
| 3 | .05 mg digested H. P. extract | 12.64 | 27.3 | 5.44 |
| 4 | 1.00 mg H. P. extract without digestion | 94.2 | 101.7 | 7.62 |
| 5 | 1.00 mg digested H. P. extract | 87.7 | 115.1 | 8.16 |

*Adrenal atrophy appears very rapidly after hypophysectomy. Therefore, in addition to examination of the sella, the adrenals were routinely weighed to make certain that hypophysectomy was complete.

the control group. However, it was significantly less only in the group receiving non-digested extract ($P=0$) and not in that receiving digested extract ($P=0.15$). On the other hand, the testes of both groups of injected males weighed significantly less than those of control animals (in both cases $P=<0.01$). Moreover, the testes of males receiving digested extract clearly weighed less than those of the group which was given non-digested extract ($P=0.02$). There is little reason to doubt that digested extract provokes the formation of "antihormone". Neither extract affected significantly the weight of the thyroid, adrenals, or seminal vesicles. Although serum was collected from these rats, circumstances beyond our control prevented tests for the presence of antigonadotropic substance.

A few remarks concerning the removal of gonadotropic extract from digested extract can be made. Alcohol (70 or 85% by volume) precipitates nearly all the hormone; the lower concentration appears as successful as the higher. (Two experiments indicate that substances insoluble in 35% alcohol contain little hormone.) In either case the pH of the digested solution of extract is adjusted to about 5.4 and the insoluble material removed before the addition of alcohol. This insoluble material was found to be inactive. The active preparations represent gravimetrically about 20% of the starting material and contain about 18% of inorganic material. Ammonium sulphate also can be used to precipitate the active principle. The latter does not dialyze through a cellophane membrane.

The procedure for the isolation of crystalline lactogenic hormone described by White, Catchpole, and Long[3] was repeated with a concentrated solution of digested horse-pituitary extract. The cautious addition of a 1% solution of $NH_4OH$ was followed by the formation of an inactive, carbon-free crystalline precipitate identified as $MgNH_4PO_4 . 6H_2O$.

Tryptic digestion of a potent extract of the urine of a patient with chorionephithelioma, under the same conditions as those employed when pituitary extract was used, completely destroyed the activity of the urinary extract.

*Summary.* In agreement with McShan and Meyer it has been found that tryptic digestion abolishes most of the luteinizing action of extracts of sheep or horse pituitary. However, large doses of digested extract were followed by the formation of some lutein tissue in the ovaries of hypophysectomized immature rats.

The effects of digested extracts on the testes of hypophysectomized immature male rats are described.

[3] White, A., Catchpole, H. R., and Long, C. N. H., *Science*, 1937, **86**, 82.

10348

## Studies of Blood Volume in Ergotamine Tartrate Poisoning in Rats.

J. Q. Griffith, Jr.,* B. I. Comroe and C. J. Zinn.

*From the Robinette Foundation, the Medical Clinic and the Endocrine Section of the Hospital of the University of Pennsylvania.*

McGrath[1] described gangrene of the tail in rats given ergotamine tartrate in toxic dosage. He quoted Polak[2] as having demonstrated that lumbar sympathectomy rendered the rat more susceptible to such gangrene. Rubin and Rapoport,[3] studying vascular hypertension in rats poisoned by ergotamine tartrate noted gangrene of the tail in most of their animals. None of these authors reported blood volume findings.

Albino rats were used in the present study. Blood volume was measured by the dye method of Griffith and Campbell.[4] The normal value by this method ranges between 4.0 and 5.3 cc per 100 g body weight, averaging 4.3 cc. Systolic blood pressure was measured under ether anesthesia by the indirect method of Griffith.[5] The normal blood pressure by this method does not exceed 150 mm of mercury. Ergotamine tartrate† was given subcutaneously in doses varying from 1.5 to 3.0 mg per 100 g body weight.

Six normal rats given 1.5 mg per 100 g body weight of ergotamine tartrate had normal blood volumes on the 5th and again on the 12th days. None developed gangrene of the tail. Blood pressure was not measured.

Blood volume measurements were made on 18 animals approximately 2 weeks after bilateral lumbar sympathectomy. The range was from 4.1 to 6.0 cc per 100 g, averaging 4.9. Seven of the 18 animals were above and 11 were within normal limits. Thus there is a tendency toward increased blood volume 2 weeks after bilateral lumbar sympathectomy.

Twenty sympathectomized animals and 21 normal animals were

---

* Atwater Kent Fellow in Medicine.

1 McGrath, E. J. G., *Arch. Int. Med.*, 1935, **55**, 942.

2 Polak, E. (quoted by McGrath), *Casop. lek. cesk.*, 1924, **63**, 1409.

3 Rubin, M. L., and Rapoport, M., *Arch. Int. Med.*, 1937, **59**, 714.

4 Griffith, J. Q., Jr., and Campbell, R., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 38.

5 Griffith, J. Q., Jr., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 394.

† The ergotamine tartrate was supplied through the courtesy of the Sandoz Chemical Works.

given 2.5 mg of ergotamine tartrate per 100 g body weight. Five of the normal animals and 12 of the sympathectomized animals developed gangrene of the tail. Blood volume was measured in 20 of the normal animals on the twelfth day following the injection. This was normal or slightly increased in 11 animals, and definitely reduced in 9 animals, ranging from 2.8 to 3.9 cc. All 5 animals with gangrene of the tail had lowered blood volume. Blood volume was again measured in the 8 survivors of this group on the 19th day after ergotamine tartrate. This was either normal (1 animal) or elevated (7 animals), the latter including one animal with healed gangrene. Blood volume measured on the eleventh day after the injection in 18 of the sympathectomized animals, was normal in 7 animals and reduced (2.7 to 3.9 cc) in 11 animals. All surviving animals with gangrene of the tail (10 animals) had lowered blood volume. On the 19th day after injection the 5 survivors, 4 of which had healed gangrene of the tail, had normal (2 animals) or elevated (3 animals) blood volume. Thus Polak's[2] observation that lumbar sympathectomy predisposes to gangrene of the tail was confirmed. In addition, it was found that this gangrene was regularly associated with diminished blood volume, which returned to normal or above after healing occurred.

Blood pressure was measured on the eleventh or twelfth day after injection of 2.5 mg of ergotamine tartrate per 100 g body weight in 9 normal and 8 sympathectomized animals, and again on 4 of the survivors on the 19th day. On the eleventh or twelfth day 7 were hypertensive, with blood pressures ranging from 150 to 240 mm of mercury, while 11 had normal pressures. Blood volume was normal or somewhat elevated in 7 animals, all of which had normal blood pressure. Blood volume was reduced in 10 animals, 3 of which had normal and 7 elevated blood pressures. No animal was hypertensive with normal or elevated blood volume. On the 19th day, the 4 animals which had previously had the highest blood pressures now had normal pressures and normal or slightly increased blood volume. Thus, hypertension was found to be regularly associated with diminished blood volume, which returned to normal or above when the blood pressure fell to normal.

Twelve normal animals were given 3.0 mg of ergotamine tartrate per 100 g body weight; 1 or 2 animals were used for blood pressure and blood volume measurements daily. The experiment was discontinued by the eighth day because of the death or poor condition of the animals, but it was found that the hypertension and the diminished blood volume appeared by the 6th day.

In 8 animals with diminished blood volume the rate of disappearance of the dye from the blood stream was followed. It was found to be normal. The finding of the diminished blood volume was, therefore, not an error due to delayed mixing of injected dye.

The primary vascular effect of ergotamine tartrate poisoning may be thought to be a generalized vascular spasm. This results in a diminished blood volume, which may occur alone or in association with either gangrene of the tail or vascular hypertension, or both. The localized vascular dilatation which follows bilateral lumbar sympathectomy tends to raise general blood volume. It also makes the tail more susceptible to the gangrene of ergotamine tartrate poisoning.

## 10349

## Observations on the Mode of Action of Sulfanilamide *in vitro*.

CAROLINE A. CHANDLER AND CHARLES A. JANEWAY.* (Introduced by Hans Zinsser.)

*From the Department of Obstetrics and the Department of Bacteriology and Immunology, Harvard Medical School, Boston.*

The bacteriostatic effect of sulfanilamide *in vitro* has been demonstrated by many workers and there is little doubt that it plays an important part in the action of the drug *in vivo*. The experiments reported in this paper were designed to analyze some of the factors that play a rôle in this bacteriostatic action.

*Methods.* For the sake of uniformity, a single strain was used throughout. This strain, obtained through the courtesy of Dr. Rebecca Lancefield, was a group A, type 6, mucoid, hemolytic streptococcus, designated S 43. Unless otherwise stated, the medium used was neopeptone-water[1] to which horse serum was added to a final concentration of 25%. The concentration of sulfanilamide† was 10 mg %. Bactericidal and phagocytic experiments were performed according to the technics described by Ward[2] and Ward and Lyons.[1] Defibrinated human blood containing no antibody to this particular strain was employed. In the phagocytic

* Work done under tenure of the Edward Hickling Bradford Fellowship.

1 Ward, H. K., and Lyons, C., *J. Exp. Med.*, 1935, **61**, 515.

† Sulfanilamide obtained through the courtesy of the Research Department of the Winthrop Chemical Co.

2 Ward, H. K., *J. Exp. Med.*, 1930, **51**, 675.

experiments 2½-hour cultures showing well encapsulated organisms were used. The viable bacteria were estimated by the standard methods of serial dilution (in broth), pour-plates and colony-counts.

*Results.* In an attempt to confirm the work of Lyons[3] the authors first investigated the effect on strain S 43 of repeated transfer in 1-10,000 sulfanilamide broth. No permanent change was demonstrable in the organism morphologically even after 18 transfers, nor could any evidence of attenuation be found. The strain maintained its original virulence for mice and its ability to grow in whole blood. In fact, in at least one instance, the culture that had been repeatedly transferred in sulfanilamide-broth became *more* resistant to the bactericidal effect of additional sulfanilamide than either the broth-transferred control or the original untransferred culture. In performing these bactericidal experiments various combinations of type-specific immune rabbit serum (.1 cc of 1:20) and sulfanilamide were added to the blood. It was found that the combination of immune serum and sulfanilamide was more bactericidal than either immune serum or sulfanilamide alone. The left-hand columns in Fig. 1 represent these findings graphically. The average number of organisms killed by 0.25 cc of blood in the combined series was 680,000 as compared with 10 organisms with immune serum alone and 9000 with sulfanilamide alone. The columns on the right of Fig. 1 indicate the effect of the repeated transfer of strain S 43 in sulfanilamide-broth. The first set of columns represents the sulfanilamide-transfer series (S.T.S. 43), the second, the broth-trans-

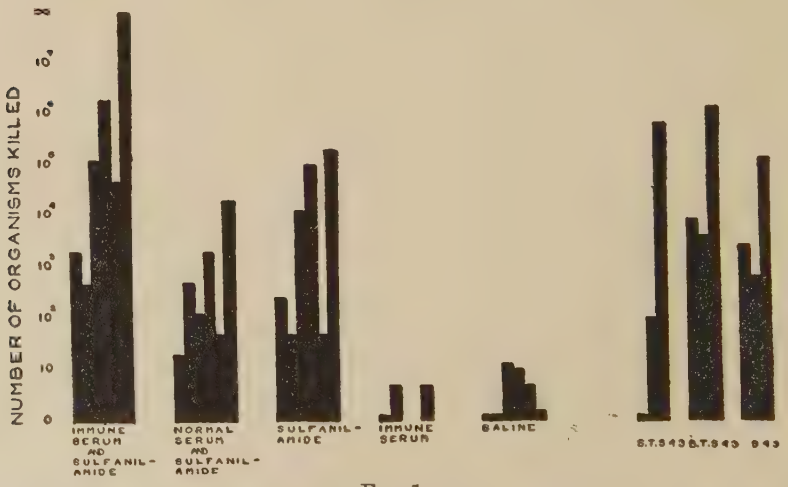



FIG. 1.

[3] Lyons, C., *Ann. Surg.*, 1938, **108**, 813.

fer series (B.T.S. 43), and the third, the original untransferred culture (S 43). From the relative heights of the columns in the 3 series it is apparent that the sulfanilamide-transferred culture showed no diminution in resistance. The fact that in making up dilutions of organisms for inoculating bactericidal tubes sulfanilamide was not added to the diluent may be the reason that the present findings fail to confirm Lyon's results.

The effect of sulfanilamide on phagocytosis was next investigated. In a concentration of 1-10,000 sulfanilamide had no demonstrable effect on phagocytosis but in more dilute solutions (1-40,000 to 1-80,000) phagocytosis was increased markedly. This observation confirms the results of Finklestein and Birkeland.[4] The probable explanation, however, is that sulfanilamide in concentrated solutions, like so many non-specific compounds[5] is somewhat toxic for leukocytes as well as bacteria but in dilute solutions is toxic only for the organisms. Such an explanation would indicate that the effect of sulfanilamide on phagocytosis is not a fundamental one and therefore does not explain its mode of action.

The final set of experiments in this series consisted of observations on the growth-characteristics of strain S 43 when exposed to sulfanilamide over varying periods. Although as previously stated no permanent morphological change was observed as a result of repeated transfer in sulfanilamide, temporary morphological alterations, similar to those noted by Gay and Clark[6] and by Lockwood[7] were noticed during the period of bacteriostasis. Capsules became larger, stained more deeply and remained on the organisms longer than in control cultures. Chains became markedly elongated and often contained as many as 50 cocci.‡

Chart 1 shows the effect of varying the size of the inoculum and Chart 2 the effect of varying the concentration of sulfanilamide with a moderately large inoculum and with a very small inoculum (initial count, not recorded on the chart, was 3 colonies per cc). These results are consistent with those of Lockwood for cultures containing peptone.

Since it was found that specific antibody greatly enhanced the killing power of sulfanilamide in human blood, the effect of this combination on the growth of the organism was determined. Chart

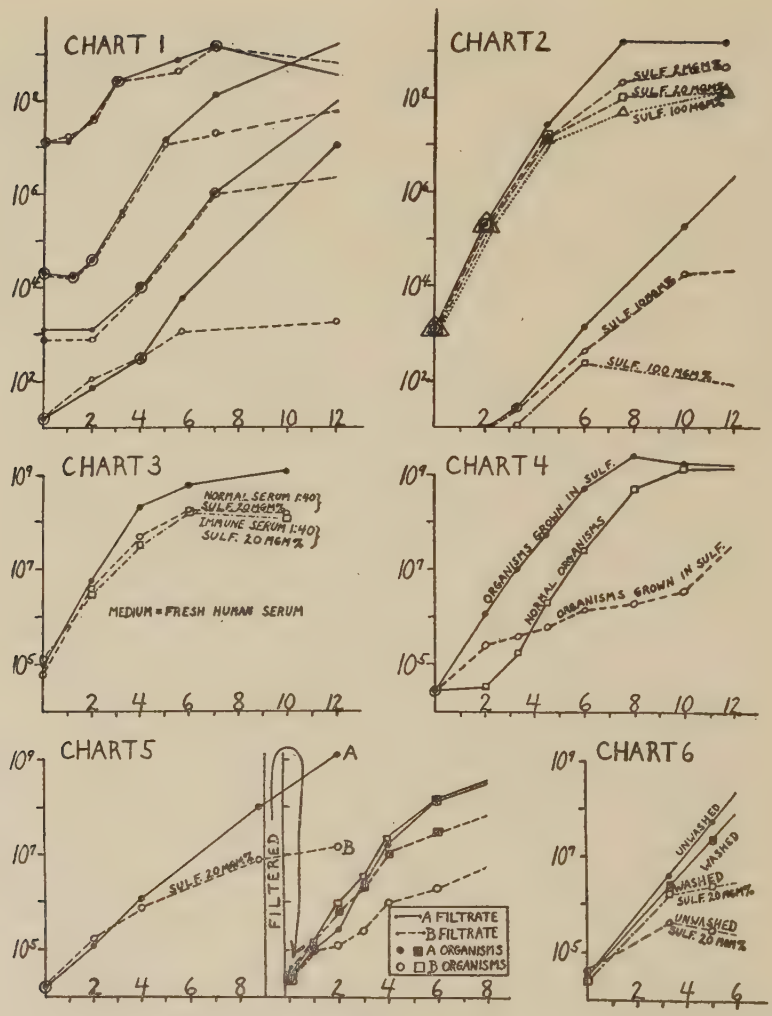
4 Finklestein, R., and Birkeland, J., *Science*, 1938, **87**, 441.

5 Tunnicliff, R., *J. Inf. Dis.*, 1931, **48**, 161.

6 Gay, F. P., and Clark, A. R., *J. Exp. Med.*, 1937, **66**, 535.

7 Lockwood, J. S., *J. Immunol.*, 1938, **35**, 155.

‡ Since colony-counts represent the number of chains, rather than number of individual cocci, this may account for some of the apparent bacteriostasis.




Growth-curves of hemolytic streptococcus, strain S43, in 25% horse-serum neopeptone-water (excluding Chart 3). Solid lines represent control cultures, broken lines cultures containing sulfanilamide (10 mg %, unless stated otherwise). Ordinates represent number of colonies per cc; abscissæ, time in hours.

3 illustrates such an experiment in which the culture medium was fresh human serum to which sulfanilamide (20 mg %) and type-specific immune rabbit serum (1:40) were added. Another experiment in which a smaller inoculum was used gave the same results.

In all of these experiments there was a period of several hours during which multiplication proceeded at a normal rate in the presence of sulfanilamide before the bacteriostatic effect became

manifest. Examination of the data of other investigators[7–12] shows such a latent period in every case and Whitby[13] has recently called attention to it. It must be kept in mind in planning and evaluating any investigation on the action of this compound. It is impossible, for example, to draw any conclusions about the effect of sulfanilamide on the oxygen-consumption of bacteria from the work of Chu and Hastings[14] because the period of observation was too short.

The following experiments were designed to show whether an alteration of the organisms or of their environment occurs during the latent period. Chart 4 shows the result of inoculating organisms, grown over night in the presence of sulfanilamide, into control and sulfanilamide-media. It is evident that the growth of such streptococci is inhibited after a very brief period of multiplication in the presence of sulfanilamide, but proceeds at a normal rate in its absence. Chart 5 records the following experiment: Two cultures, one with and one without sulfanilamide, were divided when there was definite bacteriostasis, and a portion of each was passed through a Berkefeld V filter. Each filtrate, sterile on subculture, was immediately divided, and one-half was inoculated from the first and the other from the second of the original cultures. The normal organisms were not affected by the filtrate containing sulfanilamide any more than they would have been by fresh medium containing the drug. The organisms from the sulfanilamide-culture were promptly inhibited in this filtrate, just as in the previous experiment. These results point to an alteration of the organisms themselves rather than of their environment. In order to see how firmly the sulfanilamide is bound to such altered streptococci, organisms grown in the presence of the drug were washed 3 times in plain broth in the course of an hour and inoculated into fresh media. From Chart 6 it is evident that such treatment considerably reduced their susceptibility to the action of the sulfanilamide.

*Summary.* 1. Repeated transfer of a virulent strain of hemolytic streptococcus in 1-10,000 sulfanilamide-broth does not attenuate the organism under certain experimental conditions. 2. The combined bactericidal action of antibody and sulfanilamide is greater than that

8 Colebrook, L. C., Buttle, G. A. W., and O'Meara, R. Q., *Lancet*, 1936, ii, 1323.
9 Long, P. N., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.
10 Finklestone-Sayliss, H., Paine, G. G., and Patrick, L. B., *Lancet*, 1937, i, 792.
11 Osgood, E. E., and Brownlee, I., *J. A. M. A.*, 1938, **110**, 349.
12 White, H. J., and Parker, J., *J. Bact.*, 1938, **36**, 481.
13 Whitby, L. E. N., *Lancet*, 1938, ii, 1095.
14 Chu, H. I., and Hastings, A. B., *J. Pharm. and Exp. Ther.*, 1938, **63**, 407.

of either one alone. 3. In dilute solutions sulfanilamide promotes phagocytosis, probably nonspecifically. 4. In 25% horse-serum neopeptone-water containing sulfanilamide, hemolytic streptococci form long chains and very large capsules. 5. Organisms previously grown in sulfanilamide are rapidly inhibited in fresh media containing sulfanilamide, whereas normal organisms multiply at the usual rate for several hours in the presence of sulfanilamide before bacteriostasis occurs. Evidence is presented to show that this delay is due to the formation of a loose union between the drug and the organisms rather than to any change brought about in the medium. 6. The bacteriostatic effectiveness of sulfanilamide varies directly with the concentration of the drug and inversely with the size of the inoculum. 7. The addition of specific antibody does not significantly enhance the bacteriostatic effect of the drug in fresh human serum, although it markedly increases the bactericidal effect of sulfanilamide in whole blood. 8. It is suggested that the effectiveness of a given concentration of sulfanilamide depends largely on the number of organisms present at the time when bacteriostasis begins, and this is dependent on the number of organisms inoculated and their rate of multiplication.

**10350**

## Effect of Sulfanilamide on Electrode-Potential of Hemolytic Streptococcal Cultures.

CHARLES L. FOX, JR.,* BERNARD GERMAN† AND CHARLES A. JANEWAY. (Introduced by Hans Zinsser.)

*From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.*

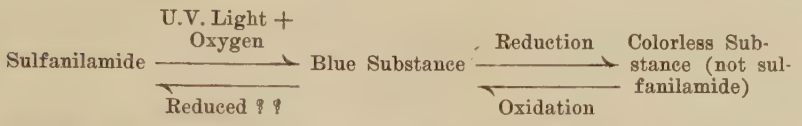
Further study[1] of the blue substance[2] formed by the photoxidation of aqueous sulfanilamide has shown that this substance is the oxidized form of a reversible system:

* Work done under tenure of the Moritz Rosenthal Fellowship assisted by the Emanuel Libman Fellowship Fund, Mt. Sinai Hospital, New York City.

† Work done under tenure of DeLamar Medical Student Fellowship, Harvard.

1 Fox, C. L., Jr., Cline, J. E., Ottenberg, R., *J. Pharm. and Exp. Ther.*, in press.

2 Ottenberg, R., and Fox, C. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 479.




Inasmuch as this substance is present in the blood[3] of patients receiving sulfanilamide, apparently responsible in part for their cyanosis, the possible effect of sulfanilamide in bacterial oxidation-reduction reactions[4, 9] was investigated.

Dubos[5] has shown that bacterial growth can be induced by a lowered oxidation-reduction potential and inhibited by an elevated potential. Fildes[6] similarly induced and inhibited the germination of tetanus spores. Clark, Cohen, and Cannon,[7] later Schmelkes,[8] showed that the addition of small amounts of iodine (or chloro compounds) at frequent intervals had no deleterious effects on a growing culture, provided the electrode-potential was never allowed to rise to the region positive to that of the aërobic cell. The total quantity of iodine added in this way far exceeded the amount necessary to sterilize the culture, when added at one time. Hewitt[4] has pointed out the great difference between the time-potential curves of hemolytic-streptococcal and pneumococcal cultures on the one hand and those of staphylococcal and *C. diphtheriæ* cultures on the other. These metabolic differences may be causally related to the differences in the response of streptococcal and staphylococcal infections to sulfanilamide.

In the present experiments hemolytic streptococci were grown with sulfanilamide under various controlled conditions and the effects on the potentials of bright-platinum electrodes were observed. Because thermodynamic interpretation of these potentials would be extremely hazardous, "it seems preferable to avoid for the present theoretical entanglements and to regard the *observations* as important."[7]

*Methods.* The apparatus and procedures used in the measurement of potentials were identical with those described by Zinsser and Schoenbach[10] except that the cultures (contained in smaller—20 cc—

3 Fox, C. L., Jr., and Cline, J. E., to be published.

4 Hewitt, L. F., *Oxidation-reduction Potentials in Biochem. and Med.*, 1936, 4th ed.

5 Dubos, R., *J. Exp. Med.*, 1929, **49**, 559, 575.

6 Fildes, P., *Brit. J. Exp. Path.*, 1929, **10**, 151, 197.

7 Clark, W. M., *Harvey Lectures*, 1933-34, p. 97.

8 Schmelkes, F. C., *J. Am. Water Works Assn.*, 1933, **25**, 695.

9 Michaelis, L., *Oxidation-reduction Potentials*, 1930.

10 Zinsser, H., and Schoenbach, E. R., *J. Exp. Med.*, 1933, **66**, 207.

flasks) were mixed continually by the Ridley alternate-suction method.[11] No attempt was made to exclude air. Duplicate readings in each culture were taken at suitable intervals.‡

The strain of hemolytic streptococcus, the media, the method of counting bacteria, and the sulfanilamide solutions were the same as those used by Chandler and Janeway.[12] Sulfanilamide-medium always contained 10 mg %. Neopeptone-water was freshly autoclaved and serum added just before each experiment. This medium (25% horse-serum neopeptone-water) is well buffered and repeated tests always gave a final pH of 8.0, except as described in the experiments with 1% glucose. The inoculum used was such as to give an initial count of 25,000 colonies per cc. Cultures were incubated at 37.5°C in a constant-temperature waterbath. Procedures adopted to alter the electrode-potentials are described in the appropriate paragraphs. All experiments have been performed at least twice.

*Results.* The graphs record typical experiments.

1. Sterile media with and without sulfanilamide showed no difference in the rise of electrode-potential that followed incubation.

2. Comparison between sulfanilamide- and control-cultures (Fig. 1): The Eh of both cultures remained the same for the first 9 hours, but growth only proceeded equally for 6 hours. After 6 hours, the control culture continued to multiply rapidly and its Eh value quickly dropped 55 mv after the eighth hour. On the other hand, little change occurred in either the population or the potential of the sulfanilamide-culture for the next 16 hours. When this culture was observed for a longer period (Fig. 2), after 24-30 hours there was a rapid fall in potential and a profuse multiplication of the organisms. During the next 30-hour period the Eh of the sulfanilamide-culture slowly rose to the initial level, as had the control many hours before.

3. The effect of the size of the inoculum on the electrode-potential (Fig. 2): The Eh of the heavily inoculated culture fell 70 mv to the minimum within 2 hours, whereas the potential of the lightly inoculated cultures remained stationary for the first 9 hours and then fell to a slightly lower minimum in the next 4 hours.

4. The effect of cysteine (Fig. 3): (0.1% neutralized cysteine

[11] Ridley, F., *Brit. J. Exp. Path.*, 1928, **9**, 253.

‡ The electrode-potential of chlorine is here regarded as positive, that of hydrogen as negative. The potential-reading added to the value of the calomel half-cell (+0.337 volt) is referred to as the electrode-potential, Eh.

[12] Chandler, C. A., and Janeway, C. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 179.

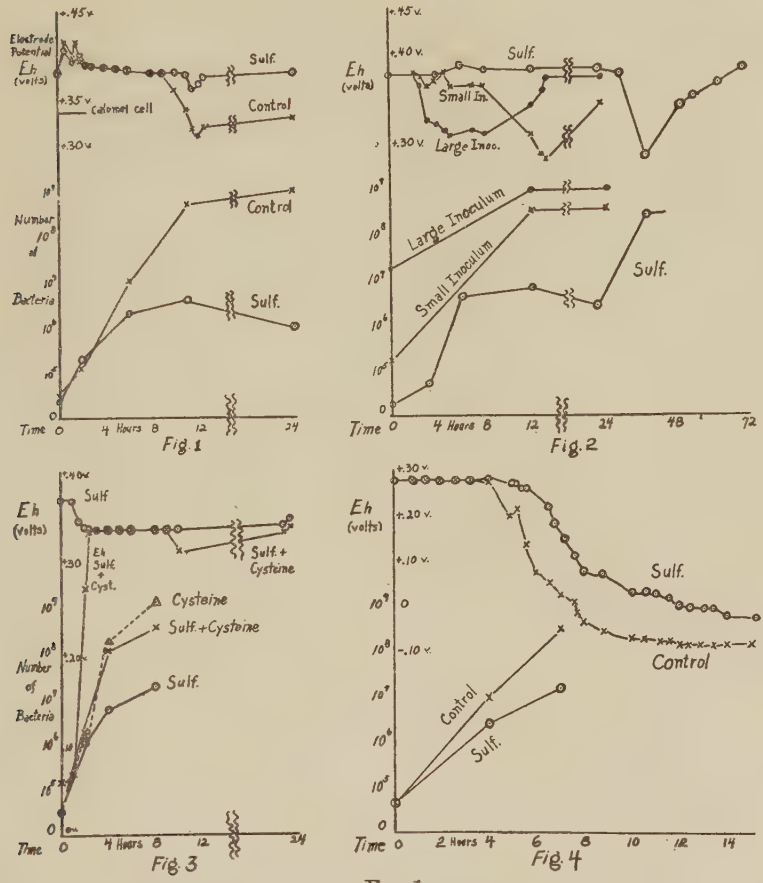



FIG. 1.

Time-potential curves and growth-curves of hemolytic streptococcus, strain S43, under various conditions. Ordinates represent number of bacteria as colonies per cc and electrode-potentials in volts; abscissæ, time in hours.

HCl was used. Potentials were measured in sulfanilamide-cultures with and without cysteine; counts were taken on these as well as on the control culture without sulfanilamide.) The initial potential of the sulfanilamide-cysteine culture was about 300 mv below that of the plain sulfanilamide-culture, but gradually rose to the same Eh level. The growth-curves show a striking reduction in the bacteriostasis by sulfanilamide in the presence of cysteine.

5. Effect of partial anaërobiosis:§ (A 1 cm layer of sterile vaseline was poured over the surface of the cultures, which were not mixed during the experiment). The Eh of both control and sul-

§ The inhibition of sulfanilamide-bacteriostasis by cysteine, anaërobiosis, and catalase is to be reported in greater detail subsequently.

fanilamide-cultures remained stationary for the first 4 hours of growth, but then the potential of the control fell 175 mv in the next 4 hours while multiplication continued at a rapid rate. The potential of the sulfanilamide-culture continued at an elevated level for 2 hours longer before falling 120 mv, while moderate growth occurred.

6. The effect of catalase: (Hewitt[4] has shown that catalase will lower the electrode-potential of a culture, presumably by decomposing $H_2O_2$[13])|| (0.1 cc of washed horse-erythrocytes were added as a source of catalase at the beginning of the experiment.) The initial potential of the sulfanilamide-catalase culture was 25 mv lower than that of the plain sulfanilamide-culture, remained so for the first 12 hours, but then gradually approached that of the control. Although more marked bacteriostasis occurred in this experiment because the organisms inoculated had been previously grown 18 hours in sulfanilamide, the sulfanilamide-culture containing catalase showed far less bacteriostasis, indicating an antagonism between sulfanilamide and catalase.¶

When liver-catalase (prepared by the method of Batelli and Stern[14]) was added to fully grown cultures the Eh promptly dropped 60 mv.

7. The effect of glucose: With 1% glucose in the media, the usual elevated initial potentials persisted in both cultures throughout a 24-hour period of observation. The pH of the control culture dropped to 5.8, that of the sulfanilamide culture persisted at 8.0. Good bacteriostasis was obtained.

8. The effect of a synthetic medium containing no protein: (Since Lockwood[16] has suggested that sulfanilamide may block the proteolytic enzymes which make it possible for the organism to grow in serum, it seemed worthwhile to study the effect of sulfanilamide in a protein-free synthetic medium, supplied by Rane and Subbarow.[17] The initial Eh of both cultures was about 160 mv below the usual level, but rose during the first 9 hours to the usual initial level,

[13] Broh-Kahn, R. H., and Mirsky, A. I., *J. Bact.*, 1938, **35**, 455; *Nature*, 1938, **142**, 153.

|| Neither the potato-benzidine method of Avery nor fresh starch-iodine paper ever yielded a positive test for $H_2O_2$ in our cultures.

¶ After this work was done, the report of Main[15] and her associates appeared on the anticatalase effect of sulfanilamide increased by ultraviolet radiation.

[14] Batelli and Stern, *Compt. Rend. Soc. Biol.*, 1934, **57**, 374.

[15] Main, E. R., Shinn, L. E., Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

[16] Lockwood, J. S., *J. Immunol.*, 1938, **35**, 155.

[17] Rane, L., and Subbarow, Y., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 837.

while practically no growth occurred. The Eh of the sulfanilamide-culture remained at this elevated level and bacteriostasis persisted; the Eh of the control culture remained elevated for 5 hours, but by the 25th hour it had fallen 170 mv, and profuse growth had occurred.

*Summary.* 1. Potentiometric measurements are reported of the electrode potentials of cultures of hemolytic streptococci growing in 25% horse-serum neopeptone-water with and without sulfanilamide. 2. The Eh of aërobic control cultures remained elevated until after active multiplication was well advanced, then rapidly fell about 50 mv. The Eh of aërobic sulfanilamide-cultures remained elevated much longer, but when bacteriostasis ceased, it also fell. 3. With a very large inoculum the Eh fell no more than with a small inoculum, but the change occurred many hours earlier. 4. The addition of cysteine or catalase, or the exclusion of air with vaseline not only lowered the potential of both control and sulfanilamide-cultures, but also definitely reduced the bacteriostatic effectiveness of sulfanilamide. 5. In the presence of 1% glucose, the Eh of both control and sulfanilamide-cultures remained elevated, and sulfanilamide was effective as a bacteriostatic agent. 6. Sulfanilamide bacteriostasis is accompanied by an elevated electrode potential; normal growth by a rapidly falling potential.

10351 P

## Effects of Light and Darkness on Activity of the Pituitary of the Rat.*

VIRGINIA M. FISKE. (Introduced by Frederick L. Hisaw.)

*From the Biological Laboratories, Harvard University.*

The effect of light on the time at which sexual maturity is attained and on the sexual cycle of a polyestrous mammal has been studied by Browman[1] and by Hemmingsen and Krarup,[2] but the nature of the change in the secretory activity of the pituitary responsible for these phenomena is as yet incompletely understood. It was with this

* Supported in part by a grant from the Rockefeller Foundation administered by Frederick L. Hisaw.

1 Browman, L. G., *J. Exp. Zool.*, 1937, **75**, 375.

2 Hemmingsen, A. M., and Krarup, N. B., *Det. Kgl. Danske Videnskabernes selskab.*, 1937, **13**, 7.

aspect of the problem in mind that the following work was undertaken.

Immature female rats were kept in darkness, in the ordinary laboratory light, and under continuous lighting from the twenty-first day of life. The rats were examined daily for vaginal introitus. The rats came into sexual activity most quickly when kept in the light, least quickly when kept in the dark (Table I). Fifty rats placed in a dark room and an equal number under light from birth, rather than when twenty-one days old, failed to give results which differed significantly from those just reported.

TABLE I.
Age at Which Vaginal Introitus Occurred in Rats Kept in the Light, the Laboratory, and in the Dark.

| No. of rats | Treatment | Age at which first vaginal rupture appeared | Age of last rats to show vaginal rupture | Age at which 50% of rats had ruptured vaginas |
|---|---|---|---|---|
| 50 | Light | 39 days | 54 days | 45 days |
| 50 | Laboratory | 42 | 69 | 51 |
| 50 | Darkness | 46 | 90 | 61 |

As soon as the vaginal membrane of the rats kept in darkness or under continuous illumination was found to be ruptured, the rats were examined by means of vaginal smears for changes in the estrous cycle. Rats kept in the light were found to have prolonged periods of estrus and somewhat lengthened periods of diestrus whereas the animals kept in the dark were continually fluctuating between estrus and diestrus and showed longer periods of metestrous smears than the normal rat (Table II).

Pituitaries from females kept in light or in darkness for 100 days

TABLE II.
Duration and Number of periods of Estrous (E), Diestrous (D), and Metestrous (M) Smears Exhibited by 50 Rats Kept in the Light or Dark Over a 60-day Interval.

| Length of period, days | Dark Room | | | Light Room | | |
|---|---|---|---|---|---|---|
| | E | D | M | E | D | M |
| 1 | 100 | 99 | 112 | 31 | 47 | 76 |
| 2 | 63 | 40 | 46 | 31 | 39 | 7 |
| 3 | 22 | 15 | 23 | 47 | 28 | 3 |
| 4 | 11 | 6 | 9 | 31 | 17 | 0 |
| 5 | 2 | 3 | 4 | 22 | 19 | 1 |
| 6 | 1 | 3 | | 2 | 8 | |
| 7 | | 2 | | 4 | 6 | |
| 8 | | | | 1 | 5 | |
| 9 | | | | 1 | 5 | |
| 10 | | | | 1 | 1 | |

were tested for their LH and FSH content by the injection of such material, following crude extraction, into 21-day-old male and female rats. It was found that the pituitaries from the females kept in the light were the more potent in producing ovarian development, whereas the pituitaries from females kept in the dark were the more potent in producing seminal vesicle growth. These results indicate that the pituitaries of females kept in the dark produce more luteinizing hormone than do those of the females kept in the light.

A study of the ovaries of females kept in the light or dark for 175 days also indicated an increased LH secretion in the dark, the ovaries being larger and heavily luteinized under such conditions, whereas those from animals in the light were almost entirely follicular.

Males kept in the light from the twenty-first day to the one hundred and seventy-fifth day of life differed from males kept in the dark for the same period in that the pituitaries, testes, and seminal vesicles of the former were larger, the pituitaries being almost twice as large, the testes 30% heavier, while the seminal vesicles were 3 times as large. The pituitaries of these animals have not yet been tested for LH and FSH.

From the results here presented it appears that the balance of the 2 gonadotropic hormones is changed under varying light conditions.

10352

## Effect of Prolonged Passage in the Chick Egg on the St. Louis Encephalitis Virus.

MARGARET G. SMITH. (Introduced by Leo Loeb.)

*From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.*

Cultivation of the virus of St. Louis encephalitis on the chorio-allantoic membrane of the chick embryo was reported by Harrison and Moore,[1] who carried one strain through 7 passages and another through 10. The titer of the virus in the chorio-allantoic membrane was not determined. They also demonstrated the presence of the virus in the brain, liver and spleen of the chick embryo, but did not determine the titer of the virus in these organs.

[1] Harrison, R. W., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 359; *Am. J. Path.*, 1937, **13**, 361.

Schultz, Williams and Hetherington[2] reported 3 series of experiments in which they carried the virus of St. Louis encephalitis on the chorio-allantoic membrane of the developing chick through 15, 16, and 22 passages, respectively. In the first 2 series, the chorio-allantoic membrane was not infective (presumably for mice) in dilutions above $10^{-1}$. In the 3rd series, however, the infective dilution increased from $1 \times 10^{-1}$ at the 5th passage to $2 \times 10^{-3}$ at the 17th passage and to $2 \times 10^{-4}$ at the 22nd passage. The chick embryo brain at the 17th passage was infective in a dilution of $2 \times 10^{-4}$, a slightly higher dilution than the membrane from the corresponding passage.

Both Harrison and Moore, and Schultz, Williams and Hetherington used for the original inoculation of the chick eggs virus grown by the flask culture method, while we have used as the original inoculum infected mouse brain virulent for mice in a dilution of $10^{-6}$. The Hubbard strain of virus used in these experiments was isolated in mice from a patient dying in the 1937 outbreak of encephalitis in St. Louis.

We have carried the virus through 68 passages on the chorio-allantoic membrane of the chick. The virus has been passed at 3 or 4 day intervals. The membranes were ground and diluted with nutrient broth pH 7.4. Amounts of 0.1 cc and 0.15 cc of a 1:3 dilution were used for transferring the virus to the chorio-allantoic membrane of eggs incubated for 10 to 12 days. For injecting eggs we used the artificial air sac technic, a modification of the Woodruff-Goodpasture method introduced by Burnet.[3]

There has been no evidence of increasing adaptation of the virus to the chorio-allantoic membrane. Membranes from early passages (1st, 2nd, 4th, and 29th) contained at 4 days enough virus to kill mice regularly when 0.03 cc amounts of $10^{-2}$ dilutions were inoculated intracerebrally. Mice receiving the $10^{-3}$ dilution died irregularly. When tested after 62 passages, the amount of virus in the membrane 4 days after inoculation was the same, killing 4 of 4 mice in a dilution of $10^{-2}$ and 1 of 4 in a dilution of $10^{-3}$. Membranes tested at 6 and 10 days after inoculation show no increase in virus over the amount present at 4 days.

The virus content of the chick embryo brain from the corresponding passage was titrated on several occasions at the same time as the membrane. In the early passages it contained enough virus on

2 Schultz, E. W., Williams, G. F., and Hetherington, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 799.

3 Burnet, F. M., Med. Research Council, Special Report, Series No. 220, 1936.

the 4th day to kill mice uniformly in dilutions of $10^{-3}$. After 62 passages the brain material 4 days after inoculation of the membrane still killed mice uniformly in dilutions only as great as $10^{-3}$. However at 10 and 11 days after inoculation, the brain seems to contain slightly more virus than at 4 days since the majority of the mice receiving the $10^{-4}$ dilution of virus die. The membrane at this time is not infective in greater dilutions that at 4 days. The presence of a slightly greater amount of virus in the embryo brain than in the chorio-allantoic membrane has been reported by Schultz, Williams and Hetherington also.

The strain of virus used for the inoculation was originally infective for mice in dilutions of $10^{-6}$, when infected mouse brain was used as the source of the virus. Presumably the virulence of the virus for mice has not been altered by passage on the egg membrane. This is demonstrated by the fact that the brains of mice, dying as the result of inoculation with the egg membrane from the 66th and from the 67th passages were still infective for mice in dilutions of $10^{-6}$. The difference in the virus concentration of the chorio-allantoic membrane and that of the mouse brain as titrated in mice is apparently due to the difference in propagation of the virus in these media rather than to any change in virulence of the egg cultured virus for the mouse.

Chick embryos apparently are little affected by the virus. The great majority of those which are not used earlier for passage or for study live until approximately the time of hatching and no gross anatomical changes have been observed. However, only a few chicks hatch successfully.

Although the amount of virus in the brain 4 days after inoculation of the membrane is sufficient to kill mice in dilutions of $10^{-3}$ and at the time of hatching in dilutions of $10^{-4}$, only slight microscopic lesions have been observed in the chick brains and even these appear in only a few chicks. The brain of one embryo 4 days after inoculation of the membrane showed two small foci of glial proliferation, one near the cortical surface and one in the midline near the ventricles. In the first focus there were also a few wandering cells, and in the meninges near by there was a cluster of mononuclear cells near a vessel. In the brains of 2 other chicks killed at the time of hatching, having been inoculated 11 days previously, several small clusters of wandering cells were seen near vessels in the meninges. No cellular infiltration about vessels in the brain has been observed.

On the 3rd or 4th day after the clear supernatant fluid from the membrane-broth mixture has been inoculated on the egg membrane,

the membrane is grossly edematous and slightly opaque. There is a very fine stippling or granulation of the surface. At a later time the edema lessens, the membranes appear thinner and have a slight but uniform opacity. Neither at 4 days nor later have we seen gross ulcerations of the membrane except when it has been suspected that the membrane was traumatized at the time of inoculation.

Microscopically, at 4 days the edema of the mesodermal layer of the membrane is apparent. There is some proliferation of fibroblasts and there are foci of cellular infiltration, chiefly mononuclear, which tend to be concentrated about blood vessels.

The ectodermal layer of cells shows focal proliferation, which may become diffuse at times. The surface cells of these foci of proliferation become necrotic, but this degeneration does not extend into the mesoderm to form an ulcer. It is the microscopic foci of ectodermal proliferation and degeneration which give the gross appearance of fine granulations. In places, small epithelial pearls extend downward into the mesodermal layer under or near the foci ectodermal proliferation and there is proliferation of fibroblasts about these downgrowths. An increase in thickness of the entodermal layers may also occur. No specific cellular inclusions have been observed.

*Summary.* After 68 passages on the chorio-allantoic membrane of the chick there is no increased proliferation of the virus on the membrane. The virulence of this virus after cultivation in eggs for 9 months has been demonstrated to be the same for the mouse as that of the original inoculum of the eggs. The virus content of the chick embryo brain is consistently slightly greater than that of the corresponding chorio-allantoic membrane. The majority of the chicks live until the time of hatching and show no gross changes attributable to the virus and only slight and inconstant microscopical changes in the brain. The membranes show a gross edema and fine granulation of the surface. Microscopically, they show chiefly edema and cellular infiltration of the mesoderm and foci of ectodermal proliferation with superimposed necrosis of the superficial layers.

10353 P

## Improved Procedure for Biological Titration of Estrogenic and Gonadotropic Hormones in Sera of Pregnant Women.

A. E. Rakoff. (Introduced by L. M. Tocantins.)

*From the Department of Obstetrics, Jefferson Medical College, Philadelphia.*

Although some studies have been reported on the concentration of estrogenic and gonadotropic substances in whole blood and serum during pregnancy, their number and accuracy have been limited by the large quantities of blood necessary for the biological tests employed.

By using unextracted sera as the test solutions, rather than extracts of blood or sera which contain only a fraction of the hormones, and mice, rather than rats, as the test animals, it was found that reliable results could be obtained with from 20 to 50 cc of blood.

*Estrogenic Hormone.* A modification of Fluhmann's technic[1] was employed. Whole or diluted serum was injected subcutaneously in 6 divided doses over a period of 3 days into castrated mice of approximately 8 weeks of age and 25 g in weight, beginning on the 8th day after oöphorectomy. The animals were sacrificed on the 4th afternoon. Biopsies were taken from the middle portion of the vagina and histologic sections prepared by a rapid technic. The least amount of serum which produced complete hyperplasia to vaginal cornification (reactions 4 to 5, Fluhmann) were regarded as containing 1 mouse unit. Also the uterine cornua were examined macroscopically for estrus, since macroscopically positive uteri indicated dosages several times the amount necessary to produce vaginal cornification.

For the initial titration 3 to 6 mice were injected with varying amounts of sera. When the approximate quantity necessary to produce cornification was found, 3 to 6 animals were injected with more closely grouped dosages. Further titrations were conducted if necessary. Six to 12 animals were generally used for each titration, while 3 to 6 animals were injected with amounts close to the finally accepted value.

The values obtained in 88 determinations on 26 pregnant women (Table I) were approximately 20 times greater than those of Smith,[2] who used ether extracts of whole blood.

1 Fluhmann, C. F., *Endocrinology*, 1934, **18**, 705.

2 Smith, M. G., *Bull. Johns Hopkins Hosp.*, 1927, **41**, 62.

TABLE I.
Serum Estrogen and Prolan in Normal Pregnancy.
(Eighty-eight determinations on 26 patients.)

| Wks of Gestation | Sera Titrated | Estrogenic Hormone | | | Gonadotropic Hormone | | |
|---|---|---|---|---|---|---|---|
| | | Avg | —Range— Low | High | Avg | Low | High |
| | | Mouse units per 100 cc of serum | | | | | |
| 6-7 | 4 | — | — | 25 | 575 | 330 | 800 |
| 8-9 | 6 | 24 | 16 | 33 | 760 | 400 | 1660 |
| 10-11 | 8 | 36 | 20 | 60 | 850 | 400 | 2000 |
| 12-15 | 10 | 43 | 16 | 100 | 960 | 300 | 2000 |
| 16-19 | 10 | 87 | 33 | 160 | 510 | 200 | 1000 |
| 20-23 | 9 | 131 | 100 | 200 | 211 | 120 | 300 |
| 24-27 | 11 | 168 | 80 | 330 | 160 | 100 | 250 |
| 28-31 | 11 | 185 | 120 | 380 | 135 | 250 | 100 |
| 32-35 | 9 | 313 | 120 | 500 | 172 | 100 | 300 |
| 36-39 | 10 | 430 | 300 | 800 | 185 | 100 | 330 |

*Gonadotropic hormone.* A modification of the Ascheim-Zondek test was employed. Whole or diluted sera were injected in 6 divided doses over a period of 3 days into infantile mice approximately 17 days old and 8 g in weight, and the animals were killed on the 5th morning. The least amount which caused the production of corpora hemorrhagica or corpora lutea in 2 of 3 animals was considered as containing one mouse unit. The ovaries were inspected with the aid of a hand lens, but any suspicious specimens were sectioned for histologic examination. It was found expedient to recognize either corpora lutea or corpora hemorrhagica as end points, because in some specimens the amount of follicle-stimulating hormone was equal to or greater than the luteinizing fraction, although it must be recognized that their significance may prove to be quite different.

The end point was most conveniently approximated by making a series of preliminary titrations using single animals for a number of widely separated dosages. Final titrations were conducted on groups of 3 animals.

A composite graph made from the titration values of 88 specimens (Table I) reveals a curve similar to that obtained from extracts of the serum by Boycott and Rowland[3] and Smith and Smith.[4] The latter workers state that they employed extracts of the sera and urines for titrations of gonadotropic substances because the unextracted material was toxic for immature animals. This difficulty was rarely experienced with the small quantities of sera required for each test during pregnancy. Whole sera yielded values many times higher than extracted specimens.

[3] Boycott, M., and Rowlands, I. W., *Brit. M. J.*, 1938, **1**, 1097.

[4] Smith, G. V., and Smith, O. W., *Am. J. Phys.*, 1934, **107**, 128.

10354

## The Prothrombin Conversion Rate in Various Species.*

E. D. Warner, K. M. Brinkhous and H. P. Smith.

*From the Department of Pathology, State University of Iowa, Iowa City.*

The rate at which thrombin forms in freshly drawn blood is known to depend upon the amounts of prothrombin and thromboplastin available. It is the purpose of the present article to show that in addition to these factors there is an element of species specificity. Even with comparable levels of prothrombin and thromboplastin, the conversion of prothrombin into thrombin is much more rapid in the plasmas of dog and rabbit than in those of man and guinea pig. This implies differences in the convertibility of prothrombin in the blood of these species. This sluggish conversion of prothrombin in man is a contributory factor in making man susceptible to a large number of hemorrhagic diseases.

The blood of dog or rabbit, when placed at once in 14 mm test tubes, clots in about 6 minutes, while the blood of man or of guinea pig, under like conditions, clots in about 11 minutes (Column 2, Table I). Data given in this same table show that this striking difference between the 2 groups is due to difference in the rate at which prothrombin is converted into thrombin.

It is easily shown that the above difference is not due to relative scarcity of thromboplastin in the blood of man and guinea pig. Thus, to each type of plasma we have added constant amounts of a very powerful preparation of thromboplastin. This addition is far in excess of the amounts normally present in clotting blood, and by this addition we have largely eliminated any relative inequality in the amount of thromboplastin. The plasma thus enriched with thromboplastin clotted quite rapidly when recalcified (Column 3), but the specific difference in speed of conversion still persisted. The plasma of dog and rabbit clotted in 9-10 seconds; those of man and guinea pig in 32-33.

In these plasmas, rich in thromboplastin, one can stop the conversion of prothrombin at varying intervals by the addition of oxalate to remove the calcium. By this technic one can study the entire curve of prothrombin conversion, and obtain further proof of the slow conversion in plasmas of man and guinea pig. Thus, in

* Aided by a grant from the John and Mary R. Markle Foundation. Funds for two research assistants were supplied by the Graduate College, State University of Iowa.

TABLE I.
Prothrombin conversion under various conditions.*

| Column 1 | Column 2 | Column 3 | Column 4 | Column 5 | Column 6 | Column 7 |
|---|---|---|---|---|---|---|
| | | Undiluted plasma plus concentrated thromboplastin (lung extract) | | | Dilute plasma (5 prothrombin units per cc) plus dilute thromboplastin (lung extract) | |
| Species | Clotting time of whole blood min | Total clotting time sec | "Latent period" of thrombin formation sec | Prothrombin conversion time (85-95% complete) sec | "Latent period" of thrombin formation sec | Prothrombin conversion time (100% complete) sec |
| Dog | 6 | 9 | 4 | 23 | 10 | 90 |
| Rabbit | 6 | 10 | 4 | 30 | 21 | 90 |
| Man | 11 | 32 | 16 | 60 | 45 | 225 |
| Guinea pig | 11 | 33 | 18 | 75 | 40 | 240 |

**Reagents for Table I:* Oxalated plasma, as described previously.[3] Lung extract made by extracting ground beef lung 48 hours at 5°. Extract then cleared in centrifuge and neutralized (pH 7.3). Fibrinogen prepared as previously described,[3] but all steps carried out at 5°, using 1/5 sat. ammonium sulphate.

Column 3: Mix 5 vol. oxal. plas. + 5 vol. lung extr. + 1 vol. $CaCl_2$ (1.2%) + 4 vol. saline (0.9% NaCl).

Column 4: Same mixture as in column 3. After varying periods of incubation (0-20 sec.) add 3 vol. potassium oxalate (1.85%), and determine minimal incubation which will produce enough thrombin to form a clot.

Column 5: Repeat experiments of column 4, but incubate 15-90 seconds, then determine in each case the residual prothrombin.[2,3] Determine the incubation time required to convert 85-95% of the original prothrombin into thrombin.

Column 6: Mix 3 vol. diluted plasma (5 prothrombin units per cc) + 3 vol. lung extr. (dil. 1-16 with saline) + 2 vol. acacia (15% in saline) + 2 vol. $CaCl_2$ (1.0%) + 3 vol. saline. Incubate varying periods (0-45 sec.), add 2 vol. oxalate (1.85%) + 3 vol. fibrinogen. Determine minimal incubation which will produce enough thrombin to form a clot.

Column 7: Same incubation mixture as in column 6. Incubate varying periods (60-300 sec.). Add 3 vol. fibrinogen, but no oxalate. Determine shortest incubation period which will give a maximal yield of thrombin.

Column 4, it is seen that in these species thrombin did not appear in the mixture in measurable amounts until 16-18 seconds had elapsed after the addition of calcium to initiate the clotting process. In the plasmas of dog and rabbit, this "latent period" was only 4 seconds, however. The species difference persists throughout the entire period of prothrombin conversion. In Column 5 are data which show that in plasmas of man and guinea pig 60-75 seconds must elapse before prothrombin conversion is 85-95% complete; in plasmas of dog and rabbit, on the other hand, only 23-30 seconds were required to bring about this degree of conversion.

Several years ago Quick also made the observation that, on adding thromboplastin, rabbit plasma clots rapidly, while human plasma

clots slowly. He ignored the possibility that there might be a difference in the ease with which prothrombin is converted into thrombin in the 2 species, and he assumed, instead, that there is a 5-fold difference in amount of prothrombin to account for the difference in clotting time.[1] He proposed, in fact, to use these accelerated clotting times as a method for determining the relative amounts of prothrombin in various plasmas. We have developed, instead, a 2-stage titration procedure for prothrombin determinations,[2, 3] and with it we have eliminated conversion speed as a variable. With this method we have shown that the prothrombin level is almost identical in the 2 species.[4] Quick's clotting time test is of value to the clinician as a presumptive test of prothrombin activity, but for purposes of research a 2-stage method must remain the standard for the quantitative determination of prothrombin.

The slow conversion of prothrombin of man and guinea pig can also be demonstrated under a different set of circumstances. If the plasma be diluted as a preliminary step, one might expect to eliminate the effect of inhibitory substances which could affect the conversion rate. Antithrombin, for example, loses most of its activity even with moderate dilution. In experiments cited in Columns 6-7, Table I, we have diluted the plasma of all 4 species 35-70 fold prior to adding calcium and thromboplastin. The plasma of guinea pig normally contains about 185 units of prothrombin per cubic centimeter; that of man 295 units; that of rabbit 310, and that of dog 350 units.[4] In making the 4 dilutions, saline was added until each mixture contained 5 prothrombin units per cubic centimeter. On adding calcium and thromboplastin each received further dilution and the final level was 1 unit per cubic centimeter. Thus, in addition to diluting inhibitors, we have also completely eliminated variations in the amount of prothrombin. Under these conditions the prothrombin conversion was still found to be more rapid in the plasmas of dog and rabbit than in those of man and guinea pig. The "latent period" of thrombin formation was 10-21 seconds in the plasmas of dog and rabbit; 40-45 in those of man and guinea pig. Pro-

1 Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501; Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282; Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

2 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

3 Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

4 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296.

thrombin conversion was complete in 90 seconds in the first 2 species; in 225-240 seconds in the last 2.

One is perhaps not justified in concluding that the prothrombin of one species is chemically different from that of another. One might assume, instead, that other substances could vary in such a way as to favor conversion in one species or impede conversion in another. It is significant, however, that partial purification of the prothrombin by ammonium sulphate fractionation does not eliminate the difference. The globulin precipitate, rich in prothrombin, is largely free of the known inhibitors of clotting.[2, 5] In experiments with dialyzed solutions of the globulin fraction we have found that the prothrombin conversion rate is still far more rapid in the canine than in the human material.

The difference in conversion rate between the species studied is not referable to any difference in the type of thromboplastin used, for in all cases the thromboplastin was obtained by saline extraction of ground beef lung. We have experiments to show that essentially identical results are obtained on using thromboplastin derived from dog lung, or by the use of homologous thromboplastins in each case. On using cephalin as a thromboplastin we have obtained slower conversion in every case, but here, too, the conversion was almost twice as rapid in plasmas of dog and rabbit as in those of man and guinea pig.

We have performed similar experiments to those of Table I on a wide variety of other vertebrates, including cats, rats, fowls, turtles and fish. When the plasmas were diluted to identical prothrombin concentrations, the conversion rate of the prothrombin almost always approximates that of dog prothrombin. The sluggish conversion in the plasma of man and guinea pig must be considered to be a handicap in the control of hemorrhage, for hemostasis depends upon covering the denuded surface promptly with a film of fibrin and platelets. If a clot is slow in forming, the blood is washed away before an effective clot can form. Many other factors influence the tendency to bleed, but the unfavorable factors, when multiple, are additive.

*Summary.* The rate at which prothrombin is converted into thrombin is much slower in the plasmas of man and guinea pig than in those of other vertebrates studied. The sluggish conversion of prothrombin in man is no doubt a factor which aggravates hemorrhagic diseases.

[5] Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

10355

# Negative Effect of Chronic Morphinism on the Anorexia Characteristic of Vitamin $B_1$ Deficiency.*

GEORGE R. COWGILL.

*From the Laboratory of Physiological Chemistry, Yale University, New Haven.*

Certain observations made by Dr. Henry G. Barbour on dogs receiving repeated doses of morphine suggested that the anorexia exhibited by such animals might be due to some effect of the continued morphine administration on the organism's requirement for vitamin $B_1$. The perfection of a technic for studying in dogs the anorexia characteristic of lack of this vitamin made it possible to study this suggested relationship experimentally.

The method employed has been used successfully for many studies from this laboratory[1] and therefore need not be described here. Certain details pertinent to the investigation here reported should be mentioned, however. Our *Casein III*[2] $B_1$-free diet was used as the basal ration. At the beginning of a feeding trial the animal received several large daily doses of either dried yeast or wheat germ in order to "saturate" the tissues with the vitamin and in this way obviate any possible effect of the previous dietary regime. The number of days between cessation of such vitamin administration and the appearance of the characteristic anorexia was then noted, and the effect of a given dose of test substance in restoring the urge to eat then observed. Since the pure vitamin was not available at the time, several products were used. Although these differed in origin, they were alike in that they were good sources of vitamin $B_1$. Their values in this respect were determined by separate assay on pigeons.[3] The test substances were dried yeast,† wheat germ,‡ and an adsorbate similar to the international standard (1934) but made with Lloyd's reagent instead of ordinary fullers earth.§ In

* The expenses of this study were defrayed by grants from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry, American Medical Association, and from the Research Funds of the Yale School of Medicine.

1 These are reviewed by Dann, M., and Cowgill, G. R., in the *Arch. Int. Med.*, 1938, **62**, 137.

2 Cowgill, G. R., *Am. J. Physiol.*, 1923, **66**, 164.

3 Block, R. J., Cowgill, G. R., and Klotz, B. H., *J. Biol. Chem.*, 1932, **94**, 765.

† From the Northwestern Yeast Company, Chicago, Ill.

‡ Embo, a product of General Mills, Inc., Minneapolis, kindly supplied by Dr. C. H. Bailey, Director of Research.

§ Kindly supplied by Dr. H. W. Rhodehamel, Director of Research Laboratories, Eli Lilly Company, Indianapolis, Ind.

a few instances a concentrate,|| which could be given parenterally when necessary, was tried.

After each of the 3 widely different sources of the vitamin had been tested on 7 dogs under "normal" conditions, the animals were fed a stock diet and morphine administration begun.¶ The initial dose was 10 mg per kilo, which was increased as tolerance permitted, first to 20 mg per kilo, and finally to 30 mg per kilo. When tolerance for this greatest dose was so well developed that the dogs appeared to enjoy "normal" health, the animals were again fed the artificial $B_1$-deficient diet, the tissues saturated with vitamin $B_1$ as described above, and the same sources of the vitamin retested for their effects on the anorexia. Finally, wherever possible, following a long period during which the drug had been withdrawn and the animals appeared to have returned to an essentially "normal" state, the tests were repeated. These final trials were not possible in all cases because many dogs died suddenly for reasons not evident on gross examination or at autopsy.

The results yielded no evidence that chronic morphinism *per se* increases the dog's requirement for vitamin $B_1$.

10356 P

## Influence of Ingestion of Raw and Desiccated Pancreas Upon Blood Lipids During Infection.*

ALBERT V. STOESSER.

*From the Department of Pediatrics, University of Minnesota, Minneapolis.*

It has been shown that there is a definite lowering of the levels for the plasma lipids at height of an acute infection as compared with the values obtained during convalescence.[1] There is, however, a tendency for the lipid content of the blood plasma to rise very

|| Made according to the method of Stuart, E. H., Block, R. J., and Cowgill, G. R., *J. Biol. Chem.*, 1934, **105**, 463, and kindly furnished by Dr. H. W. Rhodehamel.

¶ I wish to thank Dr. Henry G. Barbour of the Laboratory of Pharmacology for supervising the morphinization of these animals.

* This work was supported by a grant from the Medical Research Fund of the University of Minnesota.

1 McQuarrie, Irvine, and Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1281.

slowly and to remain below the normal range during the period of recovery as long as any infection is still present. This condition of the plasma lipids during an illness in the child has been found to be quite similar to the changes which have been observed by Chaikoff and Kaplan in the blood lipids of depancreatized dogs maintained with insulin.[2, 3]

The depancreatized dogs developed a moderate decrease in blood fat, a small drop in the plasma phospholipid values, and a marked fall in the total cholesterol due almost entirely to a reduction in ester cholesterol to the point of disappearance from the blood in some of the animals. The free cholesterol content of the blood serum changed very little. When raw pancreas was included in all diets fed after pancreatectomy, a rise instead of a fall in the blood lipids occurred.

Therefore it was thought to be worth while to feed fresh raw whole beef pancreas to children harboring infections with relatively long febrile periods and demonstrating subnormal lipid values during these periods in order to determine if the glandular tissue was capable of favorably influencing the disturbed lipid metabolism so that a return to normal would occur in a short period of time.

Twelve children ranging in age from 6 to 15 years were chosen for this study. Six of the subjects had acute osteomyelitis; one, septic arthritis; 4, acute empyema of the pleural cavity; and one, empyema with peritonitis. Following the first few days of the illness, 6 patients received raw pancreas 3 times daily in addition to the regular mixed diets of the hospital. The amount of pancreas given ranged from 40 g per meal for the smaller child, to 80 g for the larger child. It was continued throughout the period during which the temperature of the children was elevated and the white blood corpuscle count was above normal. A great deal of difficulty was encountered in getting the subjects to retain the raw pancreas, and therefore in the remaining 6 children, desiccated beef pancreas was employed. From 10 to 20 g was given with each meal and it was fairly well tolerated.

All the blood samples obtained before, during and after the period of pancreas feeding were drawn between 12 and 16 hours after a meal. The first sample of blood was collected just before the pancreas feeding was started and subsequent samples were obtained at approximately 2 to 3 week intervals during the period over which the raw pancreas was administered. The final sample of blood was

2 Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1935, **112**, 155.

3 Kaplan, A., and Chaikoff, I. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 606.

TABLE I.
Plasma Blood Lipids of Infection Before, During and After Ingestion of Pancreas.

| Case No. | Cholesterol | | | | | | | | Phospholipids | | | | Total Fatty Acids | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Esters | | | | Free | | | | | | | | | | | |
| | Mg per 100 cc serum | | | | | | | | | | | | | | | |
| | Children given fresh raw whole pancreas in diet. | | | | | | | | | | | | | | | |
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| 1. | 125 | 135 | 174 | 191 | 50 | 65 | 88 | 77 | 100 | 104 | 155 | 173 | 342 | 329 | 336 | 327 |
| 2. | 79 | 81 | 174 | 183 | 59 | 62 | 51 | 75 | 81 | 95 | 180 | 198 | 291 | 301 | 378 | 319 |
| 3. | 39 | 109 | 182 | 191 | 54 | 67 | 66 | 59 | 91 | 128 | 129 | 136 | 374 | 258 | 261 | 254 |
| 4. | 122 | 121 | 184 | 181 | 62 | 65 | 63 | 67 | 116 | 150 | 139 | 185 | 319 | 433 | 461 | 419 |
| 5. | 95 | 87 | 145 | 187 | 59 | 67 | 84 | 64 | 118 | 168 | 153 | 190 | 333 | 473 | 425 | 420 |
| 6. | 76 | 167 | 204 | 199 | 78 | 53 | 59 | 56 | 100 | 185 | 201 | 192 | 302 | 433 | 344 | 438 |
| Avg. | 89 | 117 | 177 | 188 | 60 | 63 | 68 | 66 | 118 | 138 | 159 | 179 | 327 | 371 | 367 | 363 |
| | Children given desiccated beef pancreas in diet. | | | | | | | | | | | | | | | |
| 7. | 93 | 121 | 141 | 162 | 59 | 51 | 50 | 59 | 133 | 166 | 170 | 198 | 258 | 300 | 331 | 428 |
| 8. | 112 | 164 | 194 | 196 | 52 | 80 | 52 | 54 | 148 | 189 | 189 | 223 | 328 | 277 | 471 | 420 |
| 9. | 106 | 102 | 104 | — | 59 | 76 | 56 | — | 128 | 159 | 151 | — | 317 | 366 | 252 | — |
| 10. | 84 | 112 | 141 | 122 | 44 | 49 | 76 | 60 | 69 | 73 | 180 | 173 | 280 | 284 | 351 | 305 |
| 11. | 83 | 125 | 144 | 165 | 86 | 90 | 84 | 88 | 141 | — | 189 | 198 | 253 | 294 | 295 | 315 |
| 12. | 96 | 71 | 167 | 170 | 71 | 85 | 74 | 70 | 129 | 130 | 147 | 159 | 252 | 259 | 318 | 319 |
| Avg. | 96 | 116 | 132 | 163 | 62 | 72 | 65 | 66 | 125 | 143 | 171 | 190 | 281 | 296 | 336 | 357 |

A—Blood sample collected before pancreas feeding started.
B— " " " during pancreas feeding period.
C— " " " at the end of pancreas feeding period.
D— " " " 10 to 14 days after pancreas feeding discontinued.

drawn 10 to 14 days after the pancreas feeding had been discontinued at the end of the febrile period. Bloor's methods were used to determine the various blood lipids.[4-7]

The results are summarized in Table I.

The feeding of fresh raw or desiccated beef pancreas appears to have some influence on the blood lipid levels during a rather long period of infection in the child. There was an increase in most of the blood plasma lipids of the children receiving the pancreas in spite of the fact that previous observations[8] have revealed that infection tends to keep the serum lipids below the normal range for fairly long periods of time. The cholesterol esters experienced a rapid rise from low levels to values within the normal range before the end of the febrile period. The only exception was in case 9 which showed no change in the cholesterol esters. The free cholesterol remained practically at the same level in all cases during the entire period of observation. The phospholipids made a fine recovery which was maintained in the face of existing infection. This is most significant in view of the fact that recent studies[9] have shown that the phospholipids are inclined to remain at low levels until well into the period of convalescence. The response of the total fatty acids to the feeding of the pancreas was, however, not uniform. In some subjects there was a rather rapid rise to higher levels; in others, very little change took place, and in a few, an actual fall in the total fatty acids occurred.

The apparent favorable influence of the beef pancreas may be due to the presence of pancreatic lipase in the raw product with consequent better absorption of the fat in the child's diet. Furthermore, the suggestion has been made that the favorable response of the blood plasma lipids to the ingestion of whole pancreas may be caused by the fat added to the diet in the fairly large servings of pancreas with each meal. However, the 6 cases which were given the desiccated beef pancreas did not receive any added fat. The latter product had been prepared by drying whole pancreas at about body temperature and extracting the fat with gasoline.† These children showed

4 Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

5 Bloor, W. R., and Knudgson, Arthur, *J. Biol. Chem.*, 1916, **27**, 107.

6 Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

7 Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

8 Stoesser, A. V., and McQuarrie, Irvine, *Am. J. Dis. Chil.*, 1935, **49**, 658.

9 Stoesser, Albert V., *Am. J. Dis. Child.*, 1938, **56**, 1215.

† Furnished through the courtesy of the Eli Lilly and Company Research Laboratories.

the same changes in the blood lipids as those receiving the raw whole pancreas. The unknown pancreatic blood lipid factor or factors referred to by Chaikoff and Kaplan can, therefore, still be considered the subject of further investigation.

10357

## Calcium Factor in Quantitative Determination of Prothrombin.

ARMAND J. QUICK.

*From the Department of Pharmacology, Marquette University School of Medicine, Milwaukee.*

The method developed by the author for the determination of prothrombin has on account of its simplicity won wide clinical interest. Its reliability as an index of the bleeding tendency in jaundice has been repeatedly confirmed. Nevertheless, any test based on the use of biological materials presents well recognized difficulties. In the method as originally described,[1] the thromboplastin was a source of trouble because of its variability in potency, but after the writer[2] had perfected a method for making a preparation which was stable and had a high and uniform potency, satisfactory results have been obtained. It was, therefore, surprising to learn that Stewart and Pohle[3] using the method in its present form on normal bloods found: "that the results were extremely variable." They ascribe their difficulties to the concentration of the calcium chloride solution. In the test a 0.025 M solution is used. Assuming that blood contains 10 mg of calcium, a 0.0075 M solution would be equivalent to the concentration of sodium oxalate present in the plasma. One can see that a definite excess of calcium is employed. It was recognized by the author[4] that calcium beyond a certain concentration has an inhibitory action on the coagulation time. The reason a 0.025 M solution was chosen was as follows: The prothrombin test is an outgrowth of the method originally developed at the Fifth Avenue Hospital for the determination of the clotting time of recalcified plasma and in this procedure a 0.025 M solution was found most

1 Quick, A. J., *J. Biol. Chem.*, 1935, **109**, lxxiii.

2 Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

3 Stewart, J. K., and Pohle, F. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 532.

4 Quick, A. J., *J. Immunol.*, 1935, **29**, 87.

TABLE I.
Effect of Concentration of Calcium Chloride on Clotting Time in Determination of Prothrombin.

| Molar conc. of $CaCl_2$ | *Coagulation time in seconds | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| .1 | 42 | 43 | 42 | 45 | 51 |
| .05 | 15 | 15 | 15 | 15 | 17 |
| .033 | 12 | 12 | 12 | 13 | 15 |
| .025 | 11½ | 11½ | 11½ | 11½ | 12½ |
| .020 | 11½ | 11½ | 11½ | 11½ | 12½ |
| .010 | 11 | 11 | 11 | 11 | 11 |
| .005 | 10½ | 10½ | 10½ | 10½ | 10½ |
| .0025 | 10 | 10 | 10½ | 10 | 10½ |
| .00125 | 11½ | 12½ | 10½ | 11½ | 12 |
| .000625 | 18 | 36 | 25 | 20 | 18 |

*Blood was obtained from normal human subjects.
The thromboplastin was prepared by mixing 0.3 g of dehydrated rabbit brain (acetone method) with 5 cc of 0.85% sodium chloride solution, and incubating at 50°C for 10 minutes. The supernatant liquid, obtained either by very slow centrifugation or spontaneous sedimentation of the coarse particles, was used.

satisfactory. Since this determination also has clinical value[5] it was considered wise for the sake of simplicity to retain the same calcium chloride solution for both methods.

One can see from Table I that if a satisfactory preparation of thromboplastin is used, the calcium concentration can be varied over a wide range without reducing the clotting time more than one second. No imperative need, therefore, exists for altering the strength of the calcium solution. It must be recognized, furthermore, that the values of the coagulation time in terms of prothrombin concentration have been obtained on the basis of a 0.025 M calcium chloride solution and if a different concentration of this reagent is adopted a new curve must be worked out.

The finding of Stewart and Pohle that the clotting time of the test can be slightly shortened by reducing the strength of the calcium chloride reagent is correct, but they are not justified in attributing their poor results to improper recalcification. They are right when they state that the test can only be reliable if there are no variables except the prothrombin, but they are grossly illogical when in the next statement they propose that for each determination the amount of calcium be so adjusted that a minimal coagulation time is obtained. Calcium is not a variable in the test and must not be made one. The concentration of calcium chloride was arbitrarily fixed at 0.025 M, but a different strength could have been chosen and the values of the clotting time in terms of prothrombin concentration determined for that particular calcium chloride solution.

5 Quick, A. J., Central Soc. Clin. Res., 11th meeting, 1938.

With the concentration of calcium constant, any increase in coagulation time must be due to a decrease in the prothrombin of the plasma or to interfering factors.

The inhibitory action of excess calcium chloride is probably not due to salt action, but specifically to the calcium ion itself as Aggeler and Lucia[6] suggested. This behavior of calcium deserves more careful study especially to determine the nature of its anticoagulant action. It does not appear to be an antithrombic action, since calcium in this concentration does not inhibit the action of thrombin.

10358

## Influence of Sulfanilamide and Related Compounds Upon Oxidation-Reduction Potentials of the Hemolytic Streptococcus.*

JOEL WARREN, JULIA A. STREET AND HERBERT E. STOKINGER.
(Introduced by F. P. Gay.)

*From the Departments of Bacteriology and of Biological Chemistry, College of Physicians and Surgeons, Columbia University, New York.*

In an effort to secure information as to the mechanism of the action of sulfanilamide upon the streptococcus we have measured the effect of this drug and related compounds upon the oxidation-reduction potentials of the hemolytic streptococcus. An important relation between bacteriostasis and oxidation-reduction levels was pointed out by Dubos,[1] who noted that the growth of the streptococcus and the pneumococcus was inhibited if the Eh of the system was maintained above a critical level by the introduction of suitable dyestuffs, as oxidized indophenols.

The "Holman" strain of hemolytic streptococcus (Gay) was adopted for this investigation. Freshly seeded cultures which contained 280,000 organisms per cc at the beginning of the experiment, were observed over a period of growth for 72 hours. Beef-infusion broth with 1% peptone (Bacto), pH 7.4-7.6, was used throughout. The sulfanilamide was supplied by the Winthrop Chemical Company.

Potentials were measured against 26 gauge platinum-coil electrodes. All cultures and the calomel half-cell were kept at 37.0 ± 0.5°C. A Leeds and Northrup, Type No. 7660, vacuum-tube po-

6 Aggeler, P. M., and Lucia, S. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 11.

* Aided by a grant from the Dr. Philip Hanson Hiss, Jr., Memorial Fund.

1 Dubos, R., *J. Exp. Med.*, 1929, **49**, 575.

tentiometer was used for most of the measurements, in some cases the Leeds and Northrup recording potentiometer being utilized. Suitable switch-arrangements enabled readings to be made upon 8 tubes at once. Control tubes containing no drug were set up and measured simultaneously with the sulfanilamide-containing tubes, the potentials of such control preparations being regarded as base-lines for any one experiment.

Uninoculated sterile broth containing sulfanilamide exhibited higher oxidation-reduction potentials than broth without the drug. This difference was in the region of 50 millivolts. (Fig. 1.) From this it can be seen that the drug will change the potential of sterile media. The time-potential curves obtained for streptococcal cultures were consistently higher when they contained sulfanilamide in a concentration of 1:10,000. This elevation was detectable immediately and reached a maximum at about the tenth hour. Maximal levels were about 80 to 100 mv more positive than cultures without the drug and this difference was maintained for 72 hours.

Since this difference in potential could be ascribed to a diminished growth rate of the organism, and possibly to less reduction, sulfanil-

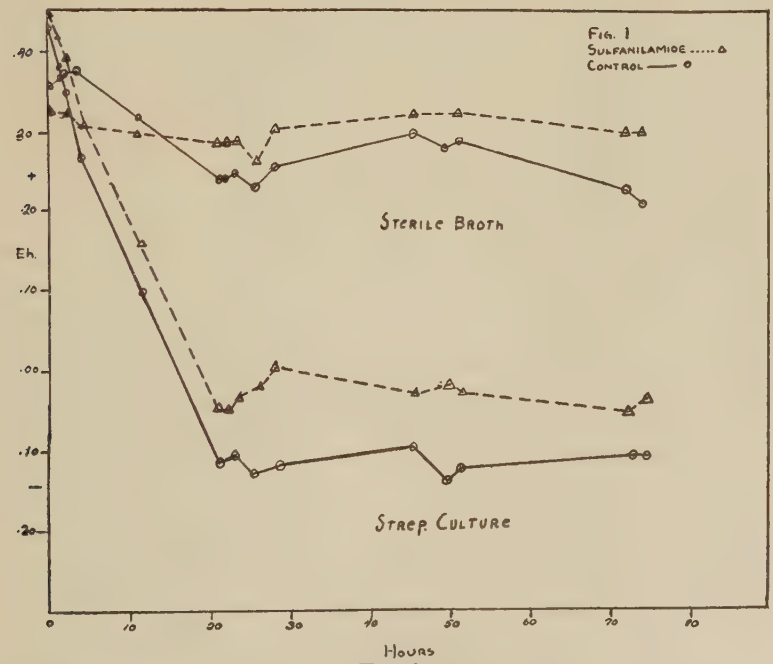



FIG. 1.

Time-potential curves of 2 typical experiments showing the elevation of Eh produced by sulfanilamide.

amide was added to cultures which had grown for 21 hours. This caused an immediate rise in E.M.F., some cultures rising as much as 210 mv, others less than 50. Experiments in which the cocci were grown in 1% or 10% rabbit-serum broth yielded similar results, although the differences in potential were not as marked. The addition of 1% M/15 phosphate buffer, pH 7.44, failed to change the effects of the sulfanilamide upon the culture-potentials.

These findings suggested that the elevations in potential induced by sulfanilamide might be decreased or even eliminated by anaërobic cultivation of the streptococci or by the addition of reducing agents to the culture. Such proved to be the case, for there was no difference between the potentials of anaërobic cultures that contained or did not contain sulfanilamide; neither was there any difference in potential in cultures grown in 0.05% cysteine broth. In such media sulfanilamide, as shown by plate counts, was non-bacteriostatic.

Several compounds related to sulfanilamide with and without therapeutic action were studied for their effects upon the oxidation-reduction potential-curves of growing streptococci. The results are summarized in Table I.

TABLE I.

| Compound | Bacteriostatic action at conc. of 1:10,000 | Effect on culture potential |
|---|---|---|
| Aniline | None | None |
| Benzenesulfonamide | " | " |
| *p*-Acetyl sulfanilamide | " | " |
| Na sulfanilyl sulfanilate | " | " |
| Disodium 4-sulphamido-phenyl-2-azo-7 acetylamino-1-hydroxynaphthalene-3,6,disulphonate ("Prontosil") | " | " |
| *p*-Aminobenzenesulfonamide | Present | Raises potential |
| 2-sulfanilamido pyridine | " | " " |
| Na formaldehyde sulphoxalate sulfanilamide | " | " " |

Prontosil is inactive *in vitro* unless it is reduced to the sulfanilamide before addition to the culture (Feinstone, Bliss, Ott, and Long[2]). This would account for the lack of potential increase encountered with this drug.

Of the series studied only those compounds which are agreed to possess bacteriostatic activity increased the oxidation-reduction potential of hemolytic streptococci over the normal level. This rise in Eh was not primarily dependent upon the number of living organisms as the drug also raises the potential of sterile broth or of previously grown cultures immediately upon addition of the compound.

[2] Feinstone, W. H., Bliss, E., Ott, E., Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

This effect could be eliminated when increased reducing-conditions are maintained in the culture. This might explain the resistance of anaërobic streptococci to sulfanilamide (Colebrook and Purdie[3]), but will not account for the alleged action of the drug upon sporulating anaërobes (Spray[4]).

The means whereby sulfanilamide produces these elevations in potential are at present obscure. It has been suggested by Main, Shinn, and Mellon[5] that the drug interferes with catalase-activity and that an accumulation of peroxides follows. However, this fails to account for the rise of potential occurring in plain broth. That sulfanilamide itself may poise the system at some critical Eh is doubtful and our attempts to measure its Eo, if any exists, have been unsuccessful.

A third possibility, which we are inclined to accept, is that sulfanilamide inactivates enzymic systems in combination with sulfhydryl or other groups normally responsible for the attainment of highly negative reduction-potentials. Such groups are presumably also present in the peptone-broth, the reduction-potential of which is rendered more positive by the drug. This hypothesis is under investigation.

The fact that sulfanilamide maintains a high Eh level in the medium, be it blood, broth, etc., may account for some of the observed properties of its therapeutic action. Lockwood[6] finds that peptone inhibits its bacteriostatic action. Peptones contain reducing agents which, as we have shown, will antagonize the oxidative effects of the drug. Lockwood, Coburn, and Stokinger[7] reported the lack of good therapeutic results when using sulfanilamide in closed, necrotic lesions as compared with diffuse inflammations. The decreased local Eh and anaërobiosis will partially account for this.

On this basis also we might expect sulfanilamide to enhance the action of humoral antibacterial factors, as reported by Branham and Rosenthal[8] for the meningococcus and pneumococcus, for Tillett and Stock[9] found that the bactericidal action of normal human serum upon the streptococcus operated at an optimum if reducing conditions were avoided.

---

3 Colebrook, L., and Purdie, A. W., *Lancet*, 1937, **2**, 1237.

4 Spray, R. S., *J. Lab. Clin. Med.*, 1938, **23**, 609.

5 Main, E., Shinn, L. E., Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

6 Lockwood, J. S., *J. Immunol.*, 1938, **35**, 155.

7 Lockwood, J. S., Coburn, A., and Stokinger, H. E., *J. A. M. A.*, 1938, **111**, 2259.

8 Branham, S., and Rosenthal, S. M., *U. S. Pub. Health Reports*, 1937, **52**, 685.

9 Tillett, W. S., and Stock, C., *J. Exp. Med.*, 1937, **66**, 617.

*Summary.* The addition of sulfanilamide to sterile broth or to cultures of the hemolytic streptococcus is accompanied by an elevation of the oxidation-reduction potential. This rise can be eliminated when the cocci are grown anaërobically or in cysteine broth. The addition of serum will slightly diminish this effect. Compounds related to sulfanilamide but lacking in bacteriostatic action are without effect upon the culture potentials. The relation of these potential effects to the action of the drug in streptococcal infections is discussed.

10359 P

## Heteroplastic Transplantation of the Hypophysis Between Different Species of Ambystoma.*

RAYMOND F. BLOUNT.

*From the Department of Anatomy, University of Minnesota.*

The differences in metamorphic activity of the axolotl *(Ambystoma mexicanum)* and the related species, *A. tigrinum,* are well known. That the thyroid of *A. mexicanum* will metamorphose thyroidless frog tadpoles although not able to metamorphose the axolotl itself was shown by Swingle.[1] It is also known through the work of Uhlenhuth and Schwartzbach[2] and others that the axolotl can be metamorphosed by anterior pituitary substances. The establishment of the pituitary's influence upon the thyroid-releasing mechanism makes it probable that the difficulty is in this relationship between these glands. The question arises as to which gland is primarily different. Witschi[3] joined axolotl and tigrinum embryos in parabiosis. These pairs metamorphosed when they were normal but did not when the tigrinum was hypophysectomized, thus showing that the pituitary gland of the tigrinum was responsible for the metamorphosis. The tigrinum thyroid was, however, present and must be considered. Bytinski-Salz[4] interchanged the pituitaries of these 2 species at the orthotopic position. His results showed no effects of the interchange. He concluded that the *Ambystoma tigrinum* with

* This work has had aid from the Graduate School Research Fund of the University of Minnesota.

1 Swingle, W. W., *J. Exp. Zool.*, 1922, **36**, 397.

2 Uhlenhuth, E., and Schwartzbach, S., *Brit. J. Exp. Biol.*, 1927, **5**, 1.

3 Witschi, E., *Anat. Rec.*, 1933, **55**, Suppl., 88.

4 Bytinski-Salz, H., *J. Exp. Zool.*, 1935, **72**, 51.

an *A. mexicanum* hypophysis will metamorphose and that conversely the axolotl with a tigrinum hypophysis will remain neotenous. This he explained by assuming that the pituitary does not act independently and that the connections between the hypophysis and the thyroid are not quantitatively fixed, but are probably controlled by another factor within the host. It would seem that the simplest explanation of his results would be on the basis of degeneration of the transplant and regeneration of the host's hypophysis. This is made more probable by the rest of his results and he admits that one can not tell the glands of the two species apart histologically.

In the present experiments the differences between the hypophysis of *Ambystoma tigrinum* and *A. mexicanum* have been brought out by comparing the results of heteroplastic and homoplastic transplants. These embryonic transplants have been made heterotopically in order that there may be no question as to which species the graft belongs. These transplants have been made in a manner described before.[5] The differences in results have been noted in pigmentation, growth and metamorphic activity.

Pigmentation of the *A. mexicanum* with an extra pituitary of its own species is definitely increased, but with an extra gland of tigrinum the melanophores show much greater expansion.

The growth changes in the axolotl are moderate under the influence of additional axolotl pituitary tissue. There is a definite slowing of growth and a shortening of gills and narrowing of tail fin and other such changes which result from additional pituitary tissue, as shown earlier.[6] However, the *A. mexicanum* with tigrinum grafts often shows extreme changes. The reduction of growth is very marked and the animal is very short and broad. The tail is specially affected and the limbs are very short and stubby. The lower jaw may protrude. These extreme individuals have not lived to metamorphic age.

In these experiments the metamorphic activity is definite. None of the animals carrying additional axolotl glands has shown any tendency to metamorphose although kept for many months. The surviving axolotls carrying tigrinum hypophyses were not extreme individuals. Of these, 2 have completely metamorphosed. They underwent their metamorphosis in a slightly longer time and somewhat later than tigrinum controls. The pituitary of *Ambystoma maculatum* as a transplant brought 2 other axolotls to metamorphic stasis. It is interesting that here the donor species is a much smaller

[5] Blount, R. F., *J. Exp. Zool.*, 1932, **63**, 113.

[6] Blount, R. F., *J. Exp. Zool.*, 1935, **70**, 131.

animal and that this stasis is known to occur with inadequate hormone supply. The graft content of these animals has not yet been studied in comparison with that of the individuals which did not metamorphose.

The thyroids of these animals have the character to be expected from their activity. That of the normal control tigrinum shows higher epithelium and some folding of follicles following the discharge associated with metamorphosis. The control axolotl shows the characteristic low epithelium and well filled follicles. In the axolotls which metamorphosed under the influence of the tigrinum hypophysis, there is some folding of follicles and a moderately high epithelium. The animal with stasis shows low epithelium with some indications of partial discharge.

These experiments present further evidence that the hypophysis of the axolotl is responsible for the non-occurrence of metamorphosis owing to inactivity in the production of the hormone associated with the releasing mechanism of the thyroid. The hypophysis of the axolotl is also less potent in the production of hormones associated with pigmentation and certain growth factors.

## 10360

## Effect of Pregnancy Urine Extract on Lactation in the Rat.*

I. L. HATHAWAY, H. P. DAVIS, R. P. REECE AND J. W. BARTLETT.
(Introduced by C. W. Turner.)

*From the Departments of Dairy Husbandry, Nebraska Agricultural Experiment Station and New Jersey Agricultural Experiment Station.*

Numerous papers have appeared reporting success in inhibiting lactation by hormone treatment. With the exception of Nelson's[1] work on the guinea pig, these investigations have not indicated whether the inhibitory action was (1) directly on the secretory epithelium of the mammary gland, (2) by the suppression of the secretion of the pituitary hormones necessary for the maintenance of lactation or (3) by the suppression of the release of pituitary hormones required for lactation. Reece and Turner's[2] work indicated

*Contribution from the Departments of Dairy Husbandry, Nebraska Agricultural Experiment Station, Journal Series No. 225, and Journal Series paper of the New Jersey Agricultural Experiment Station.

1 Nelson, W. O., *Am. J. Anat.*, 1937, **60**, 341.

2 Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1937, 266.

that the inhibitory action of estrogen on lactation in the rat could not be attributed to the suppression of either the secretion or the release of the lactogenic hormone from the pituitary.

We were interested in attempting to determine the route of the inhibitory action of pregnancy urine extracts on lactation, the inhibitory action having been previously demonstrated by others.[3-6] We were unable to inhibit lactation materially with a pregnancy urine extract (Antuitrin-S†) and as a result the work could be carried no further. The results, however, are being recorded because it appears that factors other than hormonal ones may influence the degree of lactation inhibition following the administration of Antuitrin-S.

The first injection into the experimental lactating rats was made shortly after parturition. The number of young in the litters was fixed at 6 and daily weights were recorded. All of the animals used in this experiment were fed the following ration: yellow corn 76%; linseed oil meal 16.0%; crude casein 5.0%; ground alfalfa 2.0%; NaCl 0.5%; and $CaCO_3$ 0.5%. To this mixture 5% of butter was added after grinding. Fresh whole milk was fed daily and lettuce once each week. Fifty-two lactating rats and their litters were used in this study. The control and experimental lactating rats in the first group were sacrificed on the 22nd day of the lactation period and their pituitaries and ovaries removed and weighed. The lactating rats in the other groups were not sacrificed.

Thirteen rats were injected daily for the first 5 days of the lactation period with 100 r.u. of Antuitrin-S. The first injection was made intraperitoneally and the 4 subsequent injections were made subcutaneously. Ten lactating rats and their litters served as controls. As judged by the rate of growth of the young, there was little evidence, if any, of lactation inhibition in the experimental animals. Following weaning the rate of growth of the pups from the experimental rats was just as rapid as that made by pups nursing control animals, the average weight of the pups in both groups being 65 g when 31 days old. The average weight of the ovaries from the control and experimental lactating rats was 81 mg and 130 mg respectively. With one exception, the ovaries of the injected rats were heavier than those from the non-injected rats. The pituitaries

3 Enzmann, E. V., and Pincus, G., *Am. J. Physiol.*, 1933, **103**, 30.

4 Jongh, S. E. de, *Acta brev. Neerland*, 1933, **3**, 88.

5 Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, 1934, **18**, 237.

6 Connon, F. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 52.

† We are indebted to Dr. Oliver Kamm of Parke, Davis and Company for the Antuitrin-S used in this experiment.

from the non-injected animals averaged 12.3 mg while those from the injected rats averaged 11.6 mg.

Two rats were injected intraperitoneally daily for the first 5 days of the lactation period with 200 r.u. of Antuitrin-S. Two additional rats were given the same treatment except that the extract was injected subcutaneously. Two hundred r.u. of the hormone injected subcutaneously were no more effective than 100 r.u. injected subcutaneously. There was, however, a slight inhibition of lactation in the 2 rats injected intraperitoneally with 200 r.u. of Antuitrin-S.

Three rats were injected intraperitoneally daily for 5 days with 200 r.u. of Antuitrin-S in the morning and this dosage was repeated in the afternoon. At 21 days the pups nursing the injected rats weighed 22% less than did those nursing the control mothers. Inasmuch as a considerable volume of liquid was being injected into the experimental rats it was important to ascertain the influence of this factor on lactation. Three rats were injected intraperitoneally for 5 days with 4 cc of physiological saline in the morning and 4 cc in the afternoon. This treatment caused no inhibition of lactation.

Four rats were injected intraperitoneally daily for 5 days or less with 400 r.u. of Antuitrin-S in the morning and 400 r.u. in the afternoon. The 6 pups of one rat were all dead on the 3rd day and the 6 pups of another rat were all dead on the 4th day. The pups of the other 2 rats weighed 33% less on the 21st day than did those nursing control mothers. It is doubtful, however, if this inhibition of lactation can be attributed to hormonal action only as each injection made the females very sick, the reaction beginning 3 to 4 minutes after the injection. The inhibition was not caused by the volume of liquid injected because an equal volume of physiological saline injected into 2 additional rats elicited no such response.

The above results indicated that the injection of Antuitrin-S during the first 5 days of the lactation period would not markedly inhibit lactation. It seemed worth while to determine the effect of longer periods of injection. Four rats were injected intraperitoneally daily for 16 days with 200 r.u. of Antuitrin-S. On the 15th and 21st day the young of the injected rats weighed 27% less than the young of the control animals. These results are summarized in Table I.

These results are much in contrast to those obtained by Connon,[6] who reported marked inhibition of lactation in the albino rat following Antuitrin-S injections. It may be significant that the control average weight at 21 days reported by Connon (23.2 g) was much lower than our control average figure of 37 g.

TABLE I.
Effect of Pregnancy Urine Extract on Lactation in the Rat.

| No. of Lactating Animals | Daily dosage of Antuitrin-S, r.u. | Route of Injection | No. of young on first day | Avg wt of young on the following day of the lactation cycle, g | | | | | No. of young weaned on the 21st day | Avg wt of lactating rats when litters were weaned, g |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 5 | 10 | 15 | 21 | | |
| 10 | — | | 60 | 5.3 | 9.3 | 16.8 | 26.0 | 36.4 | 58 | 258 |
| 13 | 100 | I.P.[1] S.C. | 78 | 5.3 | 8.4 | 15.6 | 24.3 | 35.4 | 75 | 272 |
| 2 | — | | 12 | 5.0 | 8.8 | 16.5 | 24.9 | 35.1 | 11 | 216 |
| 2 | 200 | I.P. | 12 | 5.0 | 7.5 | 12.5 | 19.7 | 30.2 | 11 | 229 |
| 2 | 200 | S.C. | 12 | 5.5 | 8.9 | 15.1 | 24.4 | 35.5 | 12 | 256 |
| 3 | — | | 18 | 5.6 | 9.7 | 17.1 | 26.9 | 40.7 | 18 | 273 |
| 3 | 400 | I.P.[2] | 18 | 5.6 | 7.6 | 13.1 | 20.5 | 31.6 | 18 | 286 |
| 3 | NaCl | I.P.[3] | 18 | 5.6 | 10.0 | 18.0 | 27.7 | 39.5 | 18 | 268 |
| 3 | — | | 18 | 5.5 | 9.4 | 17.8 | 27.0 | 37.8 | 18 | 247 |
| 2 | 800 | I.P.[4] | 12 | 5.6 | 7.0 | 12.0 | 16.9 | 25.5 | 12 | 260 |
| 2 | NaCl | I.P.[5] | 12 | 5.5 | 8.7 | 16.5 | 25.1 | 35.5 | 12 | 243 |
| 3 | — | | 18 | 5.4 | 9.1 | 16.7 | 25.5 | 36.3 | 18 | 248 |
| 4 | 200 | I.P.[6] | 24 | 5.2 | 7.5 | 13.5 | 18.7 | 26.6 | 24 | 265 |

[1] I.P. = Intraperitoneal; S.C. = Subcutaneous. Five daily injections, first one intraperitoneal, followed by 4 subcutaneous. One cc of Antuitrin-S contained 100 r.u.

[2] 200 r.u. injected in the morning and again in the afternoon for 5 days.

[3] 2 cc of physiological saline A.M. and P.M. for 5 days.

[4] 400 r.u. A.M. and P.M. for 5 days.

[5] 4 cc of physiological saline A.M. and P.M. for 5 days.

[6] 200 r.u. A.M. for 16 days.

*Conclusions.* The injection of 100 r.u. of Antuitrin-S daily during the first 5 days of the lactation period caused no inhibition of lactation. Larger dosages and dosages over a longer period of time reduced the rate of growth of the young from 22 to 33%. It would appear, therefore, that one cannot markedly inhibit lactation in the rat by injecting Antuitrin-S if the lactation of the control rat is normal.

10361

## Seasonal Changes in the Testes of the Passerine Bird, *Phainopepla nitens lepida.*

JAMES E. CROUCH. (Introduced by F. M. Baldwin.)

*From the Department of Zoology, University of Southern California.*

This study was undertaken to determine the histological changes which take place in the testes of the Phainopepla during an entire year. It was hoped that such changes which were noted could be correlated in some way with the behavior of the birds.

The collection of Phainopeplas for this study was started on November 26, 1937, and has been carried on periodically up to the present time, December, 1938. The gonads were removed while still warm and fixed in Bouin's solution and then carried through the usual paraffin method. They were sectioned and stained, using Delafield's hematoxylin and eosin for some, and Heidenhain's iron hematoxylin for others.

The testes showed a gradual increase in size from a minimum of one mm on November 26, 1937, to their maximum size of 8 mm, which was recorded on May 14, 1938. While the general trend throughout the spring was upward in size some individual variations were noted.

The testes showed no rapid increase in size until March 24th, and few sperms appeared in the lumina of the tubules until May 12th. This is rather unusual for in most seasons on the desert breeding starts in February and is at its height by the end of March. Although the rains were late and it stayed cool much later than usual this year it does not seem that the reproductive cycle should have been slowed to such an extent. Yet this seemed to be the true condition, as it was further substantiated by extensive field excursions during that period.

In the winter testis (Fig. 1) the size was about 1 mm, the tunica albuginea was thick, as was also the tunica propria. The seminiferous tubules were small and there was an abundance of intertubular material, composed chiefly of connective tissue cells with possibly a

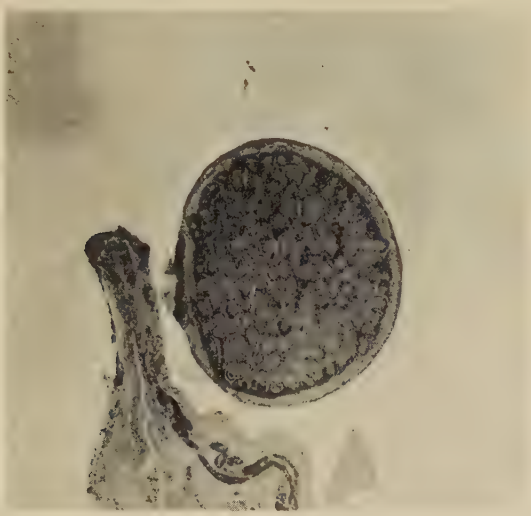


FIG. 1.
Testis of Phainopepla, winter condition. × 250. Tunica albuginea thick, lumina of tubules closed.

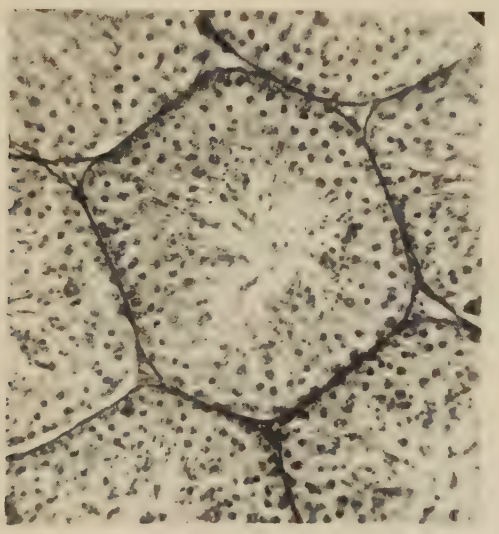


FIG. 2.
Testis of Phainopepla, intermediate condition. ×430. Tubules opening, spermatogenesis well under way.

few interstitial cells among them. The seminiferous tubules lacked lumina and there was a ring of spermatogonia along the basement membrane and a few larger nuclei just inside of these. Cell walls were not in evidence.

In Specimen 16 in the collection, taken Feb. 19, and 2 mm in length, the lumina were just opening in some of the tubules. The tunica albuginea was thinning as was also the tunica propria. Intertubular material was less abundant, but there were quite a few interstitial cells. The spermatogenic cells were more abundant.

Specimen 55 (Figs. 3 and 4) was that of an incubating bird taken June 6, 1938. It measured 6.5 mm in length. The tunica albuginea was very thin and shredding away. The tunica propria was also thin with few nuclei showing. All stages of spermatogenesis were shown in the tubules and clumps of mature sperms were present. There was little intertubular material present, only an occasional connective tissue cell or interstitial cell being apparent.

The first evidence of retrogression was seen in the vacuolation of some of the spermatogenic cells and the appearance of debris in the lumina of the tubules. This was apparent in Specimen 59, collected July 4, 1938. The specimen also showed the various stages of spermatogenesis and clumps of mature sperms arranged regularly about the lumina. Specimen 64, collected July 24, 1938, showed a further stage of regression. The size of the testis was considerably reduced, being only 4.5 mm, the tubules were reduced in proportion,

FIG. 3.

Testis of Phainopepla, active condition. × 250. Tunica albuginea thin, few nuclei in inter-tubular spaces, mature sperms in clumps around lumina of tubules.

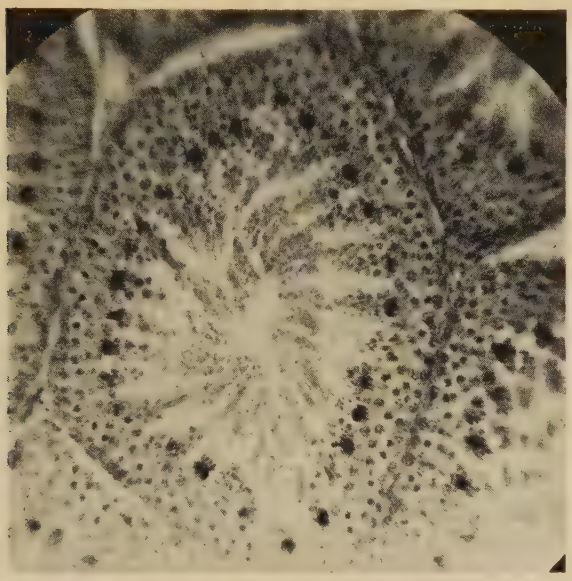


FIG. 4.
Same as Fig. 3 above. × 430.

and all stages of spermatogenesis were seen but they were scattered at random through the tubules. Complete disorganization seemed to have set in except for a regular ring of cells along the basement membrane. The tunica albuginea and tunica propria were thickening and intertubular elements were becoming prominent.

On July 30, 1938, a male was collected which was in the company of a female and 2 young. The testes of this bird measured about 2 mm in length. The tunica albuginea had reached about the thickness of the winter condition. The lumina of the tubules were almost eliminated, here and there being filled with rather large nuclei with an abundance of chromatin. The whole tubule contents was marked off by partitions or walls, and a ring of nuclei was present against the basement membrane. Intertubular elements were more abundant than in Specimen 64.

The above data on the testes of the Phainopepla bear out the studies of Rowan[1] on the Junco, and Bissonnette[2] on the English Starling.

1 Rowan, Wm., *Proc. Boston Soc. Nat. Hist.*, 1929, **39**, 151.
2 Bissonnette, T. H., *Am. J. Anat.*, 1930, **45**, 289.

10362

## Effect of Trypsin on the Virus of Trachoma.*

LOUIS A. JULIANELLE.

*From the Department of Ophthalmology, Washington University School of Medicine, St. Louis.*

In an attempt to separate the virus of trachoma from the mixture of tissues and material accompanying it when obtained from the eye, a number of chemical and physical means were studied without accomplishing the desired result.[1] While filtration is to a measure successful for this purpose, the method is unsatisfactory because of its low frequency of success, irregularity, and loss of virus.[2] The establishment, however, of the resistance of several viruses to the digestive action of trypsin[3] suggested a possible expedient for the liberation in this instance of the infectious agent from the epithelial cell, which in one way or another usually fixes the virus. Accordingly, the infectious tissues scraped from the conjunctiva of patients during operation of grattage were subjected to digestion by trypsin.

Conjunctival scrapings from both eyes of single patients were suspended in 1.5 cc of veal infusion broth, and the suspensions from different individuals were pooled and ground aseptically with mortar and pestle in the absence of abrasive. Equal portions were then removed, and to one was added an equal volume of trypsin prepared in the form of a 1% solution containing in addition 0.2% sodium bicarbonate. The trypsin did not alter the reaction sufficiently to affect the virus. The other portion of ground tissues was diluted similarly with veal infusion broth. Both mixtures were then incubated under different conditions, and the degree of digestion by trypsin was determined by smears prepared at varying intervals and stained by Wright's method. Because of the fragility of the virus, however, it was not possible to modify the basic technic as much as desired. After incubation the mixtures were shaken thoroughly and inoculated in monkeys *(M. rhesus)* by swabbing the conjunctiva of one eye, and pricking the surface of the conjunctiva of the other eye with a charged needle and then injecting subconjunctivally 0.2 cc to 0.5 cc.

* Conducted under a grant from the Commonwealth Fund of New York.

1 Julianelle, L. A., and Harrison, R. W., *Am. J. Ophthal.*, 1938, **21**, 529.

2 Julianelle, L. A., Morris, M. C., and Harrison, R. W., *ibid.*, 1937, **20**, 890.

3 Leviditi, C., and Lepine, C., *Les Ultravirus des Maladies Humaines*, 1938, Maloine, Paris.

TABLE I.
Effect of Trypsin on the Virus of Trachoma.

| Exp. No. | No. of tissues pooled | Technic of digestion | Monkeys infected with tissues | |
|---|---|---|---|---|
| | | | Undigested | Digested |
| 1 | 4 | 3 hr at 37°C | 0 of 3 | 0 of 3 |
| 2 | 2 | 2 " " 37°C | 3 " 3 | 0 " 3 |
| 3 | 4 | 30 min at 37° and 60 " " 25° | 2 " 4 | 0 " 3 |
| 4 | 2 | 30 " " 37° | 3 " 3 | 0 " 3 |

Table I describes the variations of the different experiments and illustrates the results obtained. It will be seen that 3 of 4 experiments recorded were successful. Two others were done but not recorded, since the original material was not infectious for monkeys. In the first experiment, complete digestion was allowed to take place, and this required 3 hours, an interval, however, which caused inactivation of the virus in the control material. In the remaining experiments, the period of incubation was gradually reduced to 30 minutes, which was not sufficient for complete digestion of the different cells. In each instance, however, *viz.*, digestion for 2 hours at 37°C, 30 minutes at 37°C, then 60 minutes at 25°C, and 30 minutes at 37°C, the virus was rendered non-infectious for monkeys. While the manner of conducting the experiments was necessarily limited because of the nature of the virus, it is nevertheless evident that measured by the inability to infect monkeys tryptic digestion causes inactivation of the virus of trachoma.

10363

## Distribution of Metastases in the Brown-Pearce Tumor. I. In Standard Breeds of Rabbits.*

ALBERT E. CASEY.

*From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University, New Orleans.*

During studies on the transplantation of the Brown-Pearce tumor into the testicle of the rabbit, certain variations were noted between standard breeds of rabbits.[1] These variations included (a) the incidence and volume of the primary tumors, (b) the incidence,

* This work was aided by a grant from the International Cancer Research Foundation.

1 Casey, *Am. J. Cancer*, 1937, **31**, 446.

TABLE I.

Regional Distribution of Metastases Among 202 Rabbits from Standard Breeds Injected Intratesticularly with the Brown-Pearce Tumor.

| | E | SA | F | AB | R | D | B | NZ | BV | HI | HA | BT | P | A | C | FS | L | G | SM | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Number of Animals. | | | | | | | | | | | |
| Injected | 17 | 10 | 6 | 8 | 12 | 10 | 13 | 13 | 8 | 17 | 20 | 2 | 9 | 5 | 33 | 14 | 1 | 2 | 2 | 202 |
| Negative | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 10 | 13 | 0 | 1 | 0 | 11 | 1 | 0 | 1 | 2 | 45 |
| Primary tumor | 15 | 9 | 6 | 8 | 12 | 8 | 11 | 13 | 6 | 7 | 6 | 2 | 8 | 5 | 19 | 12 | 1 | 1 | 0 | 149 |
| No metastatic foci | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 2 | 3 | 4 | 0 | 3 | 0 | 1 | 3 | 0 | 0 | 0 | 20 |
| Metastatic foci | 15 | 9 | 6 | 8 | 11 | 8 | 11 | 13 | 4 | 4 | 3 | 2 | 5 | 5 | 21 | 10 | 1 | 1 | 0 | 137 |
| Site | | | | | | | | | Number of Metastatic Foci. | | | | | | | | | | | |
| Extension to tunica | 10 | 3 | 2 | 4 | 5 | 3 | 3 | 5 | 1 | 1 | 0 | 2 | 3 | 3 | 5 | 2 | 1 | 0 | 0 | 53 |
| Spermatic cord | 11 | 7 | 4 | 6 | 8 | 4 | 8 | 9 | 2 | 1 | 1 | 1 | 3 | 2 | 15 | 6 | 1 | 0 | 0 | 89 |
| Uninoculated testicle | 2 | 0 | 1 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 8 |
| Opposite cord and tunica | 11 | 2 | 0 | 3 | 3 | 1 | 2 | 2 | 0 | 1 | 0 | 1 | 0 | 2 | 4 | 2 | 0 | 0 | 0 | 34 |
| Pelvis and bladder | 12 | 5 | 6 | 8 | 9 | 3 | 5 | 7 | 2 | 1 | 1 | 1 | 0 | 3 | 5 | 3 | 1 | 0 | 0 | 72 |
| Para-aortic tissues | 14 | 7 | 6 | 8 | 9 | 4 | 3 | 9 | 2 | 2 | 1 | 1 | 1 | 4 | 13 | 7 | 1 | 0 | 0 | 92 |
| Left peri-renal area | 11 | 5 | 5 | 7 | 4 | 2 | 2 | 3 | 2 | 1 | 0 | 0 | 2 | 2 | 4 | 3 | 1 | 0 | 0 | 54 |
| Right " " " | 12 | 6 | 3 | 5 | 6 | 3 | 4 | 4 | 2 | 1 | 0 | 0 | 1 | 3 | 6 | 4 | 0 | 0 | 0 | 60 |
| Left kidney | 15 | 8 | 6 | 8 | 9 | 7 | 9 | 8 | 3 | 1 | 1 | 1 | 4 | 4 | 14 | 6 | 0 | 1 | 0 | 105 |
| Right " | 15 | 7 | 6 | 7 | 8 | 7 | 8 | 9 | 3 | 2 | 0 | 1 | 3 | 3 | 15 | 6 | 0 | 0 | 0 | 100 |
| Left suprarenal | 8 | 2 | 6 | 3 | 4 | 1 | 4 | 6 | 1 | 0 | 0 | 0 | 1 | 2 | 6 | 3 | 0 | 0 | 0 | 47 |
| Right " | 10 | 2 | 3 | 3 | 5 | 3 | 3 | 8 | 1 | 0 | 0 | 0 | 1 | 1 | 3 | 1 | 0 | 0 | 0 | 44 |
| Parietal peritoneum | 6 | 2 | 2 | 1 | 4 | 2 | 3 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 7 | 3 | 0 | 1 | 0 | 36 |
| Serosa and mesentery | 13 | 5 | 3 | 7 | 8 | 2 | 6 | 7 | 0 | 1 | 0 | 0 | 1 | 3 | 7 | 2 | 0 | 0 | 0 | 65 |
| Omentum | 12 | 6 | 4 | 4 | 7 | 1 | 6 | 4 | 1 | 1 | 0 | 0 | 2 | 2 | 9 | 3 | 0 | 0 | 0 | 62 |
| Intestine | 8 | 1 | 1 | 3 | 2 | 1 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 25 |
| Stomach | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Liver | 11 | 4 | 4 | 5 | 1 | 3 | 1 | 6 | 1 | 0 | 0 | 2 | 3 | 2 | 4 | 1 | 1 | 0 | 0 | 49 |
| Pancreas | 1 | 0 | 2 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 10 |
| Diaphragm | 9 | 3 | 3 | 4 | 4 | 1 | 3 | 3 | 0 | 1 | 0 | 1 | 0 | 2 | 5 | 3 | 1 | 0 | 0 | 43 |
| Spleen | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Posterior mediastinum | 9 | 2 | 2 | 5 | 5 | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 5 | 3 | 0 | 0 | 0 | 37 |
| Superior " | 13 | 3 | 3 | 7 | 5 | 3 | 1 | 5 | 2 | 0 | 0 | 1 | 1 | 1 | 11 | 6 | 1 | 0 | 0 | 63 |
| Pleura, Lungs | 13 | 6 | 2 | 7 | 4 | 5 | 3 | 6 | 2 | 1 | 1 | 1 | 3 | 2 | 3 | 7 | 1 | 0 | 0 | 67 |
| Pericardium | 4 | 1 | 3 | 4 | 0 | 1 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 18 |
| Heart | 4 | 1 | 2 | 5 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 19 |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Neck, anteriorly | 5 | 2 | 2 | 5 | 2 | 1 | 2 | 6 | 1 | 0 | 0 | 1 | 0 | 1 | 3 | 1 | 0 | 0 | 0 | 32 |
| Thyroid | 3 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 9 |
| Parathyroids | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 7 |
| Muscles of tongue | 3 | 3 | 2 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 13 |
| Muscles, mastication | 6 | 6 | 1 | 1 | 3 | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 23 |
| **Mandible** | 6 | 7 | 2 | 5 | 5 | 1 | 6 | 6 | 1 | 0 | 1 | 0 | 1 | 2 | 6 | 1 | 0 | 0 | 0 | 50 |
| Gums | 8 | 6 | 2 | 5 | 6 | 1 | 6 | 8 | 2 | 1 | 1 | 1 | 0 | 2 | 5 | 3 | 0 | 0 | 0 | 57 |
| Eyes | 5 | 3 | 4 | 7 | 3 | 0 | 2 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 34 |
| Pericranium | 0 | 0 | 2 | 2 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| Meninges | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Brain | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hypophysis | 5 | 3 | 0 | 2 | 2 | 0 | 3 | 2 | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 23 |
| Nose and sinuses | 7 | 4 | 5 | 5 | 4 | 1 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 3 | 3 | 1 | 0 | 0 | 0 | 38 |
| Neck, posteriorly | 5 | 4 | 2 | 3 | 4 | 2 | 1 | 4 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 28 |
| Interscapular space | 5 | 4 | 4 | 2 | 3 | 1 | 1 | 5 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 30 |
| Muscles of scapulæ | 5 | 3 | 4 | 3 | 3 | 0 | 0 | 3 | 1 | 0 | 0 | 1 | 1 | 0 | 4 | 0 | 1 | 0 | 0 | 29 |
| Muscles, torso | 5 | 5 | 2 | 4 | 5 | 0 | 3 | 4 | 3 | 0 | 0 | 1 | 2 | 0 | 3 | 2 | 0 | 0 | 0 | 39 |
| Hypoderma, torso | 4 | 5 | 5 | 3 | 2 | 2 | 2 | 4 | 1 | 0 | **0** | **1** | **0** | 2 | 2 | 0 | 1 | 0 | 0 | 34 |
| Skin, torso | 4 | 1 | 1 | 4 | 1 | 0 | 3 | 5 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 22 |
| Muscles, thigh | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 7 |
| Metaphysis, femora | 6 | 4 | 2 | 4 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 3 | 1 | 0 | 0 | 0 | 23 |
| Metaphysis, tibiæ | 8 | 5 | 3 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 25 |
| Spinal canal | 3 | 4 | 2 | 1 | 2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 2 | 0 | 0 | 0 | 25 |
| **Totals** | 335 | 160 | 125 | 182 | 158 | 75 | 116 | 177 | 44 | 18 | 8 | 22 | 41 | 58 | 194 | 91 | 13 | 2 | 0 | 1819 |

volume, and number of the metastases, and (c) the longevity and mortality of the animals after inoculation. All the animals were males, all received the same dosage into the testicle, all were in apparent good health, there was no significant variation in age between the breeds, and the diet and housing were uniform. The variations, moreover, were consistent and were most significant among breed samples inoculated at the same time. It was not possible, therefore, to explain the variations on the basis of sex, site of inoculation, physical condition, age, diet and housing, or season.

The results, extending from a susceptibility of about 15% to a susceptibility of about 95%, seemed to be due to the inherent constitution of the pure breed strains used. The present paper reports efforts to determine whether the differences between the breeds were influenced by a selective liability of certain organs and tissues to metastatic involvement. For this purpose the protocols on 202 animals from 19 standard breeds were employed.

In each of the animals a detailed search for tumor foci was made in each of the 50 sites listed in Table I. A site containing one or more tumor nodules was designated as a single tumor focus. Forty-five animals were devoid of neoplastic growth at necropsy, 20 had primary tumors only, and 137 had metastases in one or more of the sites listed. Among the 137 animals with metastases there were 1,819 tumor foci, which amounts to approximately 20,000 tumor nodules. That an unusual and selective regional distribution of metastases had occurred was obvious from the frequency with which the kidneys, the distant lymph nodes, the jaw, the serosal surfaces, the muscles, and the skin were affected (Table I). The involvement of the regional lymph nodes, the lungs, the liver, and the suprarenal glands is common to many neoplasms, as is the infrequent involvement of the brain, the thyroid gland, the uninoculated testicle, and the spleen.

In order to ascertain whether the varying reactions of the standard breeds could be explained on the basis of selective metastasis, a statistical analysis was undertaken; the study was limited to the more commonly involved sites and to those breeds which were represented by 10 or more animals (Table II). In the tabulation are listed not only the actual number of metastatic foci encountered, but also the number which might have been expected had the distribution been due entirely to chance. It will be noted that for each breed the actual number of foci in any given location agreed fairly well with the expected values. Apparent exceptions occurred, such as the frequency of metastatic foci in the bones and skeletal muscles of

TABLE II.

Number of Metastatic Foci in Commonly Involved Sites Among Certain Breeds of Rabbits Injected Intratesticularly with the Brown-Pearce Tumor.

| | E | S | R | D | B | NZ | HI | HA | C | FS | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Actual Values. | | | | | | | |
| Regional lymph nodes | 26 | 12 | 18 | 7 | 8 | 16 | 3 | 2 | 18 | 10 | 120 |
| Extension | 21 | 10 | 13 | 7 | 11 | 14 | 2 | 1 | 20 | 8 | 107 |
| Distant lymph nodes | 39 | 20 | 21 | 9 | 8 | 25 | 0 | 0 | 22 | 13 | 157 |
| Kidneys | 30 | 15 | 17 | 14 | 17 | 17 | 3 | 1 | 29 | 12 | 155 |
| Adrenals | 18 | 4 | 9 | 4 | 7 | 14 | 0 | 0 | 9 | 4 | 69 |
| Lungs and pleura | 13 | 6 | 4 | 5 | 3 | 6 | 1 | 1 | 3 | 7 | 49 |
| Liver | 11 | 4 | 1 | 3 | 1 | 6 | 0 | 0 | 4 | 1 | 31 |
| Heart | 4 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 10 |
| Skeletal muscles | 21 | 17 | 11 | 0 | 6 | 13 | 0 | 1 | 11 | 2 | 82 |
| Bones | 20 | 16 | 5 | 1 | 7 | 7 | 0 | 1 | 11 | 3 | 71 |
| Skin | 4 | 1 | 1 | 0 | 3 | 5 | 0 | 0 | 1 | 0 | 15 |
| Totals | 207 | 106 | 100 | 51 | 72 | 125 | 9 | 7 | 128 | 61 | 866 |
| | | | | Expected Values. | | | | | | | |
| Regional lymph nodes | 28.7 | 14.7 | 13.9 | 7.1 | 10.0 | 17.3 | 1.2 | 1.0 | 17.7 | 8.5 | 120 |
| Extension | 25.6 | 13.1 | 12.4 | 6.3 | 8.9 | 15.4 | 1.1 | 0.9 | 15.8 | 7.5 | 107 |
| Distant lymph nodes | 37.5 | 19.2 | 18.1 | 9.2 | 13.1 | 22.7 | 1.6 | 1.3 | 23.2 | 11.0 | 157 |
| Kidneys | 37.0 | 19.0 | 17.9 | 9.1 | 12.9 | 22.4 | 1.6 | 1.3 | 22.9 | 10.9 | 155 |
| Adrenals | 16.5 | 8.4 | 8.0 | 4.1 | 5.9 | 10.0 | 0.7 | 0.6 | 10.2 | 4.9 | 69 |
| Lungs and pleura | 11.7 | 6.0 | 5.7 | 2.9 | 4.1 | 7.1 | 0.5 | 0.4 | 7.2 | 3.5 | 49 |
| Liver | 7.4 | 3.8 | 3.6 | 1.8 | 2.6 | 4.5 | 0.3 | 0.3 | 4.6 | 2.2 | 31 |
| Heart | 2.4 | 1.2 | 1.2 | 0.6 | 0.8 | 1.4 | 0.1 | 0.1 | 1.5 | 0.7 | 10 |
| Skeletal muscles | 19.6 | 10.0 | 9.5 | 4.8 | 6.8 | 11.8 | 0.9 | 0.7 | 12.1 | 5.8 | 82 |
| Bones | 17.0 | 8.7 | 8.2 | 4.2 | 5.9 | 10.2 | 0.7 | 0.6 | 10.5 | 5.0 | 71 |
| Skin | 3.6 | 1.8 | 1.7 | 0.9 | 1.2 | 2.2 | 0.2 | 0.1 | 2.2 | 1.1 | 15 |
| Totals | 207 | 106 | 100 | 51 | 72 | 125 | 9 | 7 | 128 | 61 | 866 |
| Chi-square = | 6.2 | 15.8 | 7.3 | 12.8 | 6.0 | 9.0 | 9.7 | 4.3 | 7.7 | 9.6 | = 88.28 |

Sable rabbits, and the scarcity with which the same sites were affected in animals of the Dutch breed.

In order to test the significance of the observed departures from the expected distribution, the chi-square test was employed.[2] The observed values were found to be so nearly like the expected values that departures as great as or greater than those observed could occur due to purely random sampling (Chi-square = 88.28, n = 100, P = 0.6, not significant†). The totals for each breed and each organ by this test likewise revealed no significant departures.

After transplantation into the testicle of 202 rabbits from 19 standard breeds, the Brown-Pearce tumor exhibited a selective and unusual distribution of metastases which affected each breed equally. The differences between various standard breeds of rabbits as to the incidence and number of the primary and metastatic tumor foci were apparently not influenced by a selective liability of certain organs and tissues to the growth of this neoplasm.

10364

## Distribution of Metastases in the Brown-Pearce Tumor. II. Effect of Fifteen Years of Transplantation.*

ALBERT E. CASEY.

*From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University, New Orleans.*

This analysis is concerned with the possible effect on the distribution of metastases of successive transplants of the Brown-Pearce tumor at monthly intervals for more than 15 years into a variety of rabbits. The data are derived from 2 series of rabbits. The first series of 191 young adult male rabbits of common mongrel type was reported by Pearce and Brown in 1923, shortly after discovery

2 Fisher, *Statistical Methods for Research Workers*, 4th ed., Oliver & Boyd, London, 1932.

† The value of P, which is 0.6, is not to be interpreted as a statistically accurate figure, as the number of items in some of the categories is too small. While not all of the categories have the recommended number of items, the value of P indicates so clearly a difference which is not significant, that this departure from the recommended minimum probably does not vitiate the conclusions.

* This work was aided by a grant from the International Cancer Research Foundation.

TABLE I.
Instances of Metastasis in Various Organs.

| Site | Actual Values | | | Expected Values | | | $\times 2/m$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Series of 1923 | Series of 1938 | Totals | Series of 1923 | Series of 1938 | Totals | Series of 1923 | Series of 1938 | Totals |
| Spread of Tumor Cells by Extension and Lymphatics. | | | | | | | | | |
| Primary spermatic cord extension | 45 | 89 | 134 | 46.38 | 87.62 | 134 | .04 | .02 | .06 |
| Retroperitoneal tissues | 47 | 92 | 139 | 48.12 | 90.88 | 139 | .03 | .01 | .04 |
| Opposite spermatic cord | 14 | 34 | 48 | 16.62 | 31.38 | 48 | .41 | .22 | .63 |
| Parietal peritoneum | 12 | 36 | 48 | 16.62 | 31.38 | 48 | 1.28 | .68 | 1.96 |
| Omentum | 30 | 62 | 92 | 31.85 | 60.15 | 92 | .11 | .06 | .17 |
| Spread of Tumor Cells by Means of Blood Vessels. | | | | | | | | | |
| Kidneys | 57 | 111 | 168 | 58.15 | 109.85 | 168 | .02 | .01 | .03 |
| Lungs | 42 | 67 | 109 | 37.73 | 71.27 | 109 | .48 | .26 | .74 |
| Suprarenals | 37 | 64 | 101 | 34.96 | 66.04 | 101 | .12 | .06 | .18 |
| Liver | 30 | 49 | 79 | 27.35 | 51.65 | 79 | .26 | .14 | .40 |
| Eyes | 18 | 34 | 52 | 18.00 | 34.00 | 52 | .00 | .00 | .00 |
| Hypophysis | 13 | 23 | 36 | 12.46 | 23.54 | 36 | .02 | .01 | .03 |
| Skin | 11 | 22 | 33 | 11.42 | 21.58 | 33 | .02 | .01 | .03 |
| Heart | 13 | 19 | 32 | 11.08 | 20.92 | 32 | .33 | .18 | .51 |
| Uninoculated testicle | 8 | 8 | 16 | 5.54 | 10.46 | 16 | 1.09 | .58 | 1.67 |
| Thyroid | 4 | 9 | 13 | 4.50 | 8.50 | 13 | .06 | .03 | .09 |
| Parathyroids | 2 | 7 | 9 | 3.12 | 5.88 | 9 | .40 | .21 | .61 |
| Spleen | 4 | 4 | 8 | 2.77 | 5.23 | 8 | .55 | .29 | .61 |
| Brain | 0 | 1 | 1 | 0.35 | 0.65 | 1 | .35 | .19 | .54 |
| Totals | 387 | 731 | 1118 | 387 | 731 | 1118 | 5.57 | 2.96 | 8.53 |

of the tumor.[1] The second series of 202 young adult male rabbits from 19 standard breeds was reported by the author in 1938.[2] The animals in both series received a single intratesticular injection of 0.3 to 0.5 cc of a saline emulsion of rabbit tumor tissue. In the first series 114 and in the second series 137 animals presented gross metastases at necropsy. The postmortem technic was the same in both series, but there were slight differences in the particular muscles, bones, and distant nodes selected for examination.

The distribution of metastases in the first and second series is presented in Table I for those sites in the body concerning which comparable data are available. The data for regional and distant metastases are segregated. Both the actual and the expected values are presented.

Neither by local extension nor hematogenous spread was there a significant variation between the actual and the expected values for the distribution of metastases. There was some evidence, furthermore, that the experimental error in the 2 series was unusually small (chi-square = 8.53, n = 17, P = 0.95). The seemingly low experimental error in the observations is borne out by the coefficient of correlation between the 2 series of +0.9828 (n = 16, P = 0.001–), a very high and significant value. The regression line is linear.

It was therefore concluded that the Brown-Pearce tumor had undergone no change in the relative distribution of metastases during 15 years of transplantation into the testicle. The distribution of metastases, furthermore, was found to be constant and characteristic for each organ.

10365

## Distribution of Metastases in the Brown-Pearce Tumor. III. Effect of Homologous Material.*

ALBERT E. CASEY.

*From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University.*

It has been shown that a single injection of a saline emulsion of 0.05-0.1 cc of Brown-Pearce tumor tissue which has been kept

1 Pearce and Brown, *J. Exp. Med.*, 1923, **38**, 347.

2 Casey, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 228.

*This work was aided by a grant from the International Cancer Research Foundation.

frozen for 10 days or longer in an anaerobic state will render rabbits susceptible to subsequent transplantation with the tumor.[1] The injection of this homologous material increases (a) the incidence and volume of the primary tumor growths, and (b) the incidence, number, and volume of the metastases, with a consequent decrease in the longevity and an increase in the mortality of the animals. The material, when injected, produces no obvious lesions, nor does it visibly affect the health of the animal. It can be desiccated, passed through a Berkefeld "V" filter,[2] and inactivated by heating to 55°C.[3] It differs biologically from the spreading factor described by Duran-Reynals.[4]

It has been shown that the relative distribution of metastases in this tumor is constant and characteristic following intratesticular inoculation, and remains unaffected by long transplantation into various breeds of rabbit.[5] The present paper concerns the possible effects of the injection of the homologous material in relation to the relative distribution of metastases.

Two groups of rabbits were used. The first consisted of 202 young adult male rabbits from 19 standard breeds, which were injected with the tumor alone.[5] The second consisted of 44 young adult male rabbits of pure and mongrel stocks, which received a single injection of 0.3 cc of the homologous material into one testicle 2 weeks before inoculation with the tumor. Both groups of animals received a single unilateral intratesticular injection of 0.3 cc of a saline emulsion of fresh Brown-Pearce tumor. The variations of breed in the 2 groups were not considered important, since this factor has been shown not to affect the relative distribution of metastases.[6] Complete necropsies were performed in the usual manner[1] on the animals which died spontaneously, and at the end of 2 months on all the animals which had survived.

The number of instances in which metastases were found in various sites are summarized in Table I, which presents both the actual and the expected values. For facilitation of the statistical analysis it was convenient to assume that the relative distribution of metastases was constant and characteristic in the 2 series, and that variations from the expected values could be attributed to ex-

1 Casey, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 816; *Am. J. Cancer*, 1934, **21**, 760.

2 Casey, *Am. J. Cancer*, 1936, **26**, 276.

3 Casey and Moragues, in press.

4 Casey, *Arch. Path.*, 1937, **23**, 741.

5 Casey, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 228.

6 Casey, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 230.

TABLE I.

Instances of Metastasis in Various Organs After Intratesticular Inoculation of the Brown-Peace Tumor.

| Site | Actual Values | | | Expected Values | | | $\times 2/m$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Controls | Treated | Totals | Controls | Treated | Totals | Controls | Treated | Totals |
| Tunic extension | 53 | 28 | 81 | 57.0 | 24.0 | 881 | .28 | .67 | .95 |
| Primary cord | 89 | 35 | 124 | 87.3 | 36.7 | 124 | .03 | .08 | .11 |
| Opposite " | 34 | 22 | 56 | 39.4 | 16.6 | 56 | .74 | 1.76 | 2.50 |
| Pelvis and bladder | 72 | 33 | 105 | 73.9 | 31.1 | 105 | .04 | .12 | .16 |
| Para-aortic tissues | 92 | 39 | 131 | 92.2 | 38.8 | 131 | .00 | .00 | .00 |
| Left perirenal area | 54 | 26 | 80 | 56.3 | 23.7 | 80 | .09 | .22 | .31 |
| Right " " | 60 | 25 | 85 | 59.8 | 25.2 | 85 | .00 | .00 | .00 |
| Parietal peritoneum | 36 | 18 | 54 | 38.0 | 16.0 | 54 | .11 | .25 | .36 |
| Serosa and mesentery | 65 | 25 | 90 | 63.4 | 26.6 | 90 | .04 | .10 | .14 |
| Omentum | 62 | 31 | 93 | 65.5 | 27.5 | 93 | .19 | .55 | .74 |
| Diaphragm | 43 | 18 | 61 | 43.0 | 18.0 | 61 | .00 | .00 | .00 |
| Posterior mediastinum | 37 | 19 | 56 | 39.4 | 16.6 | 56 | .15 | .35 | .50 |
| Superior " | 63 | 37 | 100 | 70.4 | 29.6 | 100 | .81 | 1.67 | 2.48 |
| Neck, anteriorly | 32 | 15 | 47 | 33.1 | 13.9 | 47 | .04 | .09 | .13 |
| Neck, posteriorly | 28 | 9 | 37 | 26.1 | 10.9 | 37 | .14 | .33 | .47 |
| Interscapular space | 30 | 8 | 38 | 26.8 | 11.2 | 38 | .38 | .91 | 1.29 |
| Muscles, torso | 39 | 13 | 52 | 36.6 | 15.4 | 52 | .16 | .37 | .53 |
| Hypoderma, torso | 34 | 9 | 43 | 30.3 | 12.7 | 43 | .45 | 1.08 | 1.53 |
| Visceral pleura | 67 | 33 | 100 | 70.4 | 29.6 | 100 | .16 | .39 | .55 |
| Pericardium | 18 | 6 | 24 | 16.9 | 7.1 | 24 | .07 | .07 | .24 |
| Left kidney | 105 | 34 | 139 | 97.9 | 41.1 | 139 | .51 | 1.23 | 1.74 |
| Right " | 100 | 41 | 141 | 99.3 | 41.7 | 141 | .00 | .01 | .01 |
| Lungs | 67 | 33 | 100 | 70.4 | 29.6 | 100 | .16 | .39 | .55 |
| Left suprarenal | 47 | 23 | 70 | 49.3 | 20.7 | 70 | .11 | .26 | .37 |
| Right " | 44 | 16 | 60 | 42.2 | 17.8 | 60 | .08 | .18 | .26 |
| Gums | 57 | 13 | 70 | 49.3 | 20.7 | 70 | 1.20 | 2.86 | 4.06 |
| Mandible | 50 | 12 | 62 | 43.7 | 18.3 | 62 | .91 | 2.17 | 3.08 |
| Liver | 49 | 26 | 75 | 52.8 | 22.2 | 75 | .27 | .65 | .92 |
| Nose and sinuses | 38 | 13 | 51 | 35.9 | 15.1 | 51 | .12 | .29 | .41 |
| Eyes | 34 | 9 | 43 | 30.3 | 12.7 | 43 | .45 | 1.08 | 1.53 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Muscles, scapulæ | 29 | 9 | 38 | 26.8 | 11.2 | 38 | .18 | .43 | .61 |
| Intestines | 25 | 14 | 39 | 27.5 | 11.5 | 39 | .23 | .54 | .77 |
| Spinal canal | 25 | 9 | 34 | 23.9 | 10.1 | 34 | .05 | .12 | .17 |
| Metaphysis, tibiæ | 25 | 8 | 33 | 23.2 | 9.8 | 33 | .14 | .32 | .56 |
| Metaphysis, femora | 23 | 10 | 33 | 23.2 | 9.8 | 33 | .00 | .00 | .01 |
| Hypophysis | 23 | 10 | 33 | 23.2 | 9.8 | 33 | .00 | .00 | .01 |
| Muscles, mastication | 23 | 5 | 28 | 19.7 | 8.3 | 28 | .55 | 1.31 | 1.86 |
| Skin, torso | 22 | 8 | 30 | 21.1 | 8.9 | 30 | .04 | .09 | .13 |
| Heart | 19 | 6 | 25 | 17.6 | 7.4 | 25 | .11 | .26 | .37 |
| Muscles, tongue | 13 | 4 | 17 | 12.0 | 5.0 | 17 | .08 | .20 | .28 |
| Parathyroids | 7 | 6 | 13 | 9.2 | 3.8 | 13 | .52 | 1.27 | 1.79 |
| Thyroid | 9 | 4 | 13 | 9.2 | 3.8 | 13 | .00 | .01 | .01 |
| Pericranium | 8 | 2 | 10 | 7.0 | 3.0 | 10 | .14 | .33 | .47 |
| Muscles, anteriorly, thigh | 7 | 3 | 10 | 7.0 | 3.0 | 10 | .00 | .00 | .00 |
| Spleen | 4 | 10 | 14 | 9.9 | 4.1 | 14 | 3.51 | 8.49 | 12.00 |
| Stomach | 3 | 5 | 8 | 5.6 | 2.4 | 8 | 1.21 | 2.81 | 4.02 |
| Meninges | 3 | 2 | 5 | 3.5 | 1.5 | 5 | .07 | .17 | .24 |
| Brain | 1 | 1 | 2 | 1.4 | 0.6 | 2 | .11 | .27 | .38 |
| Totals | 1868 | 785 | 2653 | 1868.0 | 785 | 2653 | 14.63 | 33.85 | 49.49 |

2 2 n — 1 0.202
2 2 m — 1 0.202

perimental errors having a random distribution. To test this hypothesis the values of $x^2/m$ (square of departures divided by expectations) were calculated and presented at the right of the table. Chi-square was 49.49, which with the degrees of freedom equal to 48 was found not to exceed what might have been expected from the foregoing assumptions ($\sqrt{2x^2} - \sqrt{2n-1} = 0.202$). It is even suggestive that the experimental error is unusually small; were it not for the values for the spleen the 2 series could be said to consist of a pattern of 2 highly correlated variables with a minimum of experimental error. The coefficient of correlation on the 2 distributions was $+0.922$ ($n = 46$, $P = 0.001-$, significant) and the regression line is linear. The only significant departure from the regression line is in the value for the spleen, in which more metastases were found among treated animals than was to have been expected. It is our belief that the original observations were not correct, and that the error occurred because animals receiving homologous material frequently had massive metastases involving the omentum and often the capsule of the spleen by extension. Such extensions probably should not be considered true metastases to the spleen. When they are eliminated, the data indicate the expected involvement for the spleen. Furthermore, when homologous material was administered to animals which were later inoculated intracutaneously in another series of experiments, no significant preponderance of splenic metastases was noted.

It is therefore concluded that injection of rabbits with an homologous material from the Brown-Pearce tumor does not affect the relative distribution of metastases following transplantation. The constancy and character of the relative distribution of metastases in this tumor is thus confirmed for the third time.[5, 6]

10366

## Distribution of Metastases in the Brown-Pearce Tumor. IV. Effects of Site of Inoculation.*

ALBERT E. CASEY AND BJARNE PEARSON.

*From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University.*

It has been shown that the distribution of metastatic foci in the

*This work was aided by a grant from the International Cancer Research Foundation.

Brown-Pearce tumor of the rabbit (a) is not disturbed by the breed of animal used for transplantation,[1] (b) has remained constant over a period of 15 years of transplantation,[2] and (c) is not affected by treating the animal with an homologous material.[3] All the inoculations in the 3 series were intratesticular and unilateral.

The present paper concerns the possible effect of intracutaneous inoculation upon this constant and characteristic distribution of metastases. To determine this effect a comparison was made of the distribution of metastatic foci in rabbits injected intracutaneously with the same distribution in rabbits injected intratesticularly.[3]

Observations were made upon 469 rabbits. Thirty-two rabbits 8 to 18 weeks old were injected intracutaneously over the right scapula, first with 0.3 cc of an homologous material, and 2 weeks later with 0.3 cc of a saline emulsion of Brown-Pearce tumor (Table I). Four hundred thirty-seven young adult rabbits were injected intratesticularly with 0.3 cc of a saline emulsion of Brown-Pearce tumor; 44 of this group also received homologous material.[3] An additional num-

TABLE I.
Metastases from Primary Tumor Growth in Skin Over Scapula in Rabbits Treated with an Homologous Tumor Material.

| Site | Metastasis | Site | Metastasis |
|---|---|---|---|
| Extension | 12 | Skin | 3 |
| Interscapular space | 8 | L eye | 4 |
| R axillary node | 13 | R eye | 5 |
| L axillary " | 2 | Ant. portion thigh muscles | 5 |
| R inguinal " | 3 | Thyroid | 1 |
| L inguinal " | 0 | Mandible | 7 |
| R kidney | 16 | Teeth | 3 |
| L " | 15 | Muscles, thorax and abdomen | 9 |
| Lungs and pleura | 10 | Heart | 2 |
| Omentum and ligaments | 2 | Muscles of mastication | 8 |
| Liver | 9 | Femora | 3 |
| Intestine | 5 | Tibiæ | 3 |
| R adrenal | 5 | Spleen | 2 |
| L " | 5 | Diaphragm | 3 |
| Muscles of scapula | 8 | Pericardium | 1 |
| Retroperitoneal space | 6 | R spermatic cord | 1 |
| Bladder and pelvis | 7 | L " " | 0 |
| L perirenal area | 7 | Pancreas | 1 |
| R " " | 5 | Parathyroid | 0 |
| Serosa | 6 | Brain and dura | 4 |
| Stomach | 3 | Hypophysis | 2 |
| Superior mediastinum | 5 | Muscles of tongue | 3 |
| Posterior " | 3 | R testicle | 2 |
| Posterior cervical region | 4 | L " | 1 |
| Anterior " " | 4 | Spinal canal | 1 |
| Pericranium | 1 | L fallopian tube | 1 |
| Nose and nasal sinuses | 3 | Parietal peritoneum | 1 |

[1] Casey, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 223.
[2] Casey, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 228.
[3] Casey, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 230.

TABLE II.

Comparison of Metastatic Foci from Testicular and Intracutaneous Inoculation of the Brown-Pearce Tumor.

| Site | Actual values | | Expected Values | | | $x^2/m$ | | |
|---|---|---|---|---|---|---|---|---|
| | Testis | Skin | Testis | Skin | Total | Testis | Skin | Totals |
| | Extension and Lymphatic Spread. | | | | | | | |
| Primary spermatic cord extension | 169 | 1 | 159.86 | 10.14 | 170 | — | — | 8.86 |
| Retroperitoneal tissues | 178 | 6 | 173.03 | 10.97 | 184 | — | — | 2.48 |
| Opposite spermatic cord | 70 | 0 | 65.83 | 4.17 | 70 | — | — | 4.49 |
| Parietal peritoneum | 66 | 1 | 63.00 | 4.00 | 67 | — | — | 2.43 |
| Omentum | 123 | 2 | 117.55 | 7.45 | 125 | — | — | 4.32 |
| Primary skin extension, right shoulder | 0 | 12 | 11.28 | .72 | 12 | — | — | 188.04 |
| Right axillary nodes | 45 | 13 | 54.54 | 3.46 | 58 | — | — | 27.57 |
| Subtotals | | | | | | 14.34 | 223.85 | 238.19 |
| | | | | | | n = 6, P = 0.001— | | |
| | Hematogenous Spread. | | | | | | | |
| Kidneys | 212 | 17 | 215.33 | 13.67 | 229 | .05 | .74 | .79 |
| Lungs | 142 | 10 | 142.94 | 9.06 | 152 | .01 | .08 | .09 |
| Suprarenals | 132 | 7 | 130.71 | 8.29 | 139 | .01 | .22 | .23 |
| Liver | 105 | 9 | 107.20 | 6.80 | 114 | .04 | .66 | .70 |
| Eyes | 61 | 6 | 63.00 | 4.00 | 67 | .06 | .94 | 1.00 |
| Hypophysis | 46 | 2 | 45.14 | 2.86 | 48 | .02 | .27 | .29 |
| Skin | 41 | 3 | 41.38 | 2.62 | 44 | .00 | .05 | .05 |
| Heart | 38 | 2 | 37.61 | 2.39 | 40 | .00 | .07 | .07 |
| Uninoculated testis | 18 | 1 | 17.87 | 1.13 | 19 | .00 | .01 | .01 |
| Thyroid | 17 | 1 | 16.93 | 1.07 | 18 | .00 | .01 | .01 |
| Parathyroids | 15 | 0 | 14.11 | 0.89 | 15 | .06 | .89 | .95 |
| Spleen | 18 | 2 | 18.81 | 1.19 | 20 | .03 | .53 | .56 |
| Brain | 2 | 0 | 1.88 | 0.12 | 2 | .00 | .18 | .18 |
| Subtotals | | | | | | 0.38 | 4.65 | 4.93 |
| | | | | | | n = 13, P = 0.98 | | |
| Totals | 1498 | 98 | 1498.00 | 95.00 | 1593 | | | |

ber of rabbits injected intracutaneously with the tumor alone are not included in this analysis because no visceral metastases were observed. (Table I.)

Many sites had too few metastatic foci for adequate statistical analysis and are therefore excluded from this comparison. The sites affected were arranged in 2 groups (Table II), according to whether spread seemed to have occurred by tissue spaces and lymphatic channels or was hematogenous. There were 7 sites in the former and 13 in the latter group. Both the actual and the expected values are presented, with their totals. To test the significance of the differences between actual and expected values, the figures $x^2/m$ were calculated.[4] The method of calculating this value has been explained previously.[3] These values are summarized as subtotals for each group of sites.

In the group of sites in which spread seemed to have occurred by tissue spaces and lymphatic channels, the metastatic foci are predominantly concentrated near the sites of inoculation, and obviously differ according to whether inoculation was intracutaneous or intratesticular, as shown by the first subtotal values at the right of the table (chi-square = 238.19, n = 6, P = 0.00001–). This is in contrast with the results reported in the preceding papers in which the distribution of metastatic foci was constant in this group of sites when the same site of inoculation was employed.

In the group of sites in which metastasis seemed to have occurred by the hematogenous route, the actual and expected values for intratesticular and intracutaneous inoculations show so little variation that almost no experimental error need be assumed (chi-square = 4.93, n = 12, P = 0.98).† The coefficient of correlation calculated upon these variables was found to be +0.9716, a value having a linear regression line and indicating a high and significant relationship (n = 11, P = 0.001–).

It was therefore concluded (a) that the relative distribution of distant metastases in the Brown-Pearce tumor is constant and characteristic, follows a definite pattern, and is independent of the site of inoculation, and (b) that spread by tissue spaces and lymphatic channels is determined by the site of inoculation.

---

4 Fisher, *Statistical Methods for Research Workers*, 4th ed., Oliver & Boyd, London, 1932.

† While not all the categories have the recommended number of items, the value of P indicates so clearly a difference which is not significant, that this departure from the recommended minimum probably does not vitiate the conclusions.

10367

# Distribution of Metastases in the Brown-Pearce Tumor. V. Comparison with Certain Tumors in Man.*

Bjarne Pearson and Albert E. Casey.

*From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University, New Orleans.*

The relative distribution of metastases from the Brown-Pearce tumor has been shown to be constant (a) for standard breeds of rabbits,[1] (b) over many years of transplantation,[2] and (c) for 2 sites of inoculation.[3]

The distribution of metastatic foci in 4 human tumors was compared with the distribution from the Brown-Pearce tumor in rabbits. The human tumors compared were primary in the cervix, breast, prostate, and rectum. The sites of metastasis chosen for comparison were the liver, lungs, kidneys, and spleen, these being sites in which fairly accurate necropsy data are usually available.

The data for the Brown-Pearce tumor were derived from a total of 393 rabbits.[2] The human data were derived from a total of 1,214 cases and were compiled from various sources. The data for carcinoma of the cervix were derived from a total of 832 necropsy reports by Albers-Schonberg,[4] McCormac,[5] Wertheimer,[6] and Pearson.[7] The data for carcinoma of the breast were derived from 74 necropsy reports by Williams[8] and Mielecke.[9] The data for carcinoma of the prostate were derived from 138 necropsy reports by Graves and Militzer[10] and Mintz and Smith.[11] The data for carcinoma of the rectum were derived from 170 necropsy reports by Brown and Warren.[12]

*This work was aided by a grant from the International Cancer Research Foundation.

1 Casey, Proc. Soc. Exp. Biol. and Med., 1939, **40**, 223.

2 Casey, Proc. Soc. Exp. Biol. and Med., 1939, **40**, 228.

3 Casey and Pearson, Proc. Soc. Exp. Biol. and Med., 1939, **40**, 234.

4 Albers-Schonberg, *Jahrb. a. Hamb. Staatskrankenanst.*, 1893-4.

5 McCormac, *Arch. Middlesex Hosp.*, 1909, **15**, 20.

6 Wertheimer, *Strahlentherapie*, 1921, **12**, 90.

7 Pearson, *Am. J. Cancer*, 1936, **28**, 31.

8 Williams, quoted by Deaver and McFarland, *The Breast, Anomalies, Diseases, and Treatment*, P. Blakiston's Sons and Co., 1917.

9 von Mielecke, *Ztschr. f. Krebsforsch.*, 1913, **13**, 505.

10 Graves and Militzer, *J. Urol.*, 1935, **33**, 235.

11 Mintz and Smith, *New England J. Med.*, 1934, **211**, 478.

12 Brown and Warren, *Surg. Gynec. and Obst.*, 1938, **66**, 611.

The material available was analyzed by means of the chi-square test to determine the homogeneity of cases of carcinoma of the cervix uteri (Table I), carcinoma of the breast (Table II), and carcinoma of the prostate (Table III). The test revealed no significant difference in the relative distributions of metastasis to the liver, lungs, kidneys, and spleen in any of the 3 series. The expected values in each table are enclosed in parenthesis. The third set of values represents $x^2/m$ or the $(\text{difference})^2/\text{expected values}$.

TABLE I.
Actual and Expected Instances of Metastasis to Liver, Lungs, Kidney, and Spleen from Carcinoma of the Cervix.

| Author | Values | Liver | Lungs | Kidneys | Spleen | Totals |
|---|---|---|---|---|---|---|
| Albers-Schonberg | actual | 77 | 23 | 10 | 7 | 117 |
| | expected | (70.68) | (27.22) | (8.69) | (10.4) | (117) |
| | $x^2/m$ | 0.57 | 0.56 | 0.20 | 1.11 | 2.53 |
| McCormac | actual | 14 | 5 | 3 | 7 | 29 |
| | expected | (17.51) | (3.26) | (2.15) | (4.16) | (29) |
| | $x^2/m$ | 0.70 | 0.93 | 0.33 | 1.93 | 3.89 |
| Wertheimer | actual | 20 | 14 | 1 | 2 | 37 |
| | expected | (22.34) | (8.60) | (2.74) | (3.29) | (37) |
| | $x^2/m$ | 0.24 | 3.39 | 1.10 | 0.50 | 5.23 |
| Pearson | actual | 11 | 5 | 1 | 2 | 19 |
| | expected | (11.47) | (4.42) | (1.41) | (1.69) | (19) |
| | $x^2/m$ | 0.02 | 0.03 | 0.11 | 0.06 | .22 |
| Totals | actual | 122 | 47 | 15 | 18 | 202 |
| | expected | (122) | (47) | (15) | (18) | (202) |
| | $x^2/m$ | 1.53 | 5.00 | 1.74 | 3.60 | 11.87 |

($n = 12$, $P = 0.5$ not significant.)†

TABLE II.
Actual and Expected Instances of Metastasis from Carcinoma of the Breast.

| Author | Values | Liver | Lungs | Kidneys | Spleen | Totals |
|---|---|---|---|---|---|---|
| Williams | actual | 20 | 8 | 2 | 1 | 31 |
| | expected | (17.33) | (9.98) | (2.10) | (1.57) | (31) |
| | $x^2/m$ | 0.41 | 0.39 | 0.00 | 0.20 | 1.00 |
| Mielecke | actual | 13 | 11 | 2 | 2 | 28 |
| | expected | (15.66) | (9.01) | (1.89) | (1.42) | (28) |
| | $x^2/m$ | 0.45 | 0.44 | 0.00 | 0.24 | 1.13 |
| Totals | actual | 33 | 19 | 4 | 3 | 59 |
| | expected | (33) | (19) | (4) | (3) | (59) |
| | $x^2/m$ | .86 | .83 | 0.00 | .44 | 2.13 |

($n = 3$, $P = 0.5$ not significant.)†

† While not all the categories have the recommended number of items the value of P indicates so clearly a difference which is not significant, that this departure from the recommended minimum probably does not vitiate the conclusions.

TABLE III.
Actual and Expected Instances of Metastasis from Carcinoma of the Prostate.

| Author | Values | Liver | Lungs | Kidneys | Spleen | Totals |
|---|---|---|---|---|---|---|
| Mintz and Smith | actual | 20 | 24 | 3 | 0 | 47 |
| | expected | (19.20) | (25.15) | (2.64) | (0.00) | (47) |
| | $x^2/m$ | 0.03 | 0.05 | 0.05 | 0.00 | .13 |
| Graves and Militzer | actual | 9 | 14 | 1 | 0 | 24 |
| | expected | (9.80) | (12.84) | (1.35) | (0.00) | (24) |
| | $x^2/m$ | 0.06 | 0.10 | 0.09 | 0.00 | .25 |
| Totals | actual | 29 | 38 | 4 | 0 | 71 |
| | expected | (29) | (38) | (4) | (0.00) | (71) |
| | $x^2/m$ | .09 | .15 | .14 | 0.00 | .38 |

($n = 2$, $P = 0.85$ not significant.)†

TABLE IV.
Actual and Expected Instances of Metastasis from Various Tumors.

| Tumor | Values | Liver | Lungs | Kidneys | Spleen | Totals |
|---|---|---|---|---|---|---|
| Brown-Pearce | actual | 79 | 109 | 168 | 8 | 364 |
| | expected | (145.78) | (114.35) | (88.84) | (15.03) | (364) |
| | $x^2/m$ | 30.59 | 0.25 | 70.53 | 3.29 | 104.66 |
| Carcinoma cervix | actual | 122 | 47 | 15 | 18 | 202 |
| | expected | (80.90) | (63.45) | (49.29) | (8.34) | (202) |
| | $x^2/m$ | 20.88 | 4.26 | 23.85 | 7.03 | 56.02 |
| Carcinoma breast | actual | 33 | 19 | 4 | 3 | 59 |
| | expected | (23.63) | (18.53) | (13.95) | (2.44) | (59) |
| | $x^2/m$ | 3.71 | 0.01 | 7.10 | 0.13 | 10.95 |
| Carcinoma rectum | actual | 57 | 38 | 4 | 4 | 103 |
| | expected | (41.25) | (32.35) | (25.14) | (4.25) | (103) |
| | $x^2/m$ | 6.01 | 0.99 | 17.87 | 0.01 | 24.88 |
| Carcinoma prostate | actual | 29 | 38 | 4 | 0 | 71 |
| | expected | (28.43) | (22.30) | (17.35) | (2.93) | (71) |
| | $x^2/m$ | 0.01 | 11.05 | 10.27 | 2.93 | 24.26 |
| Totals | actual | 320 | 251 | 195 | 33 | 799 |
| | expected | (320) | (251) | (195) | (33) | (799) |
| | $x^2/m$ | 61.20 | 16.56 | 129.62 | 13.39 | 220.77 |

The variation in the relative distribution of metastatic foci for the organs and tumors studied is presented in Table IV. The analysis is carried out in the manner of the preceding tables. The values for the actual and expected number of metastatic foci and for $x^2/m$ are presented. Chi-square for the entire series was equal to 220.77, a total which indicates more variation than should occur by chance ($n = 12$, $P = 0.00001-$). The variations were also significant for each tumor and each organ studied.

The significant variation between the 5 tumors as to the relative distribution of metastasis to the liver, lungs, kidneys and spleen con-

sisted of (a) an unusually high and significant incidence of metastasis to the kidneys in animals affected with the Brown-Pearce tumor and a relatively low incidence of metastasis to the kidneys in all 4 human tumors studied; (b) an unusually low incidence of metastasis to the liver in the Brown-Pearce tumor of the rabbit and a relatively high incidence of metastasis to the liver in carcinoma of the cervix and of the rectum; (c) an unusually high incidence of metastasis to the lung in carcinoma of the prostate and a probable significantly low incidence of metastasis to the lung in carcinoma of the cervix; (d) a relatively high incidence of metastasis to the spleen in carcinoma of the cervix. The seemingly low incidence of metastasis to the spleen from the Brown-Pearce tumor and from carcinoma of the prostate is only suggestive.

*Summary.* It is demonstrated by an appropriate statistical method that the relative distribution of distant metastases from the Brown-Pearce tumor in the rabbit is unique, and that the relative distributions of distant metastases from certain tumors of man are both constant and characteristic.

**10368**

## A Pigmented Adenoma of the Intermediate Lobe in a Rat Chronically Treated with Oestrin.

C. S. McEUEN,* H. SELYE AND J. B. COLLIP.

*From the Department of Biochemistry, McGill University, Montreal.*

Chromophobe adenomata of the anterior lobe have repeatedly been observed in rats[1, 2] and mice[3, 4, 5] following chronic treatment with oestrogenic preparations. In one of our cases we also noted a small adenoma of the intermediate lobe. We did not feel justified in concluding that this tumor was produced by the oestrin treatment because it occurred only in one case. Yet the fact that tumors in the anterior lobe and the mammary gland, and metaplasia of the uterine epithelium were seen in several animals of this series made

* Finney-Howell Research Foundation Fellow.

1 McEuen, C. S., Selye, H., and Collip, J. B., *Lancet*, 1936, 775.

2 Zondek, Bernhard, *Lancet*, 1936, 776.

3 Cramer, W., and Horning, E. S., *Lancet*, 1936, 247.

4 Cramer, W., and Horning, E. S., *Lancet*, 1936, 1056.

5 Lacassagne, A., and Nyka, W., *C. r. Soc. Biol.*, 1937, **126**, 1112.

it appear worth while to mention this occurrence and to reproduce a microphotograph of the intermediate lobe adenoma.[1]

Recently we have observed another similar case in a hooded rat which was fed a crude ether extract of pregnancy urine (from which most of the oestriol had been removed) in doses approximately corresponding to 100-150 International units of oestrin per day, from the 24th to the 880th day of life. Autopsy revealed a large pituitary tumor weighing 84 mg. As it was thought that the tumor was a chromophobe anterior lobe adenoma, which is a frequent occurrence in rats chronically treated with oestrin, and as nothing macroscopically abnormal was noted at autopsy other than the enlargement of the pituitary, only this and the testes were preserved for histo-

FIG. 1.
Intermediate lobe tumor containing dark pigment granules.

logical examination. It was noted that the testes were unusually large for an oestrin-treated animal, especially if we consider its advanced age. They weighed 3.790 g, and on microscopic examination proved to be normal. The mammary glands were well developed and contained milk cysts. It was also noted that despite its age, the animal was unusually vigorous and well nourished. It weighed 354 g.

Histological examination of the hypophysis showed that most of the enlargement was due to an enormous adenoma of the intermediate lobe. The tumor obviously emerged from the pars intermedia and contained typical melanin granules such as are usually found in the normal intermediate lobe cells of colored rats (Fig. 1).

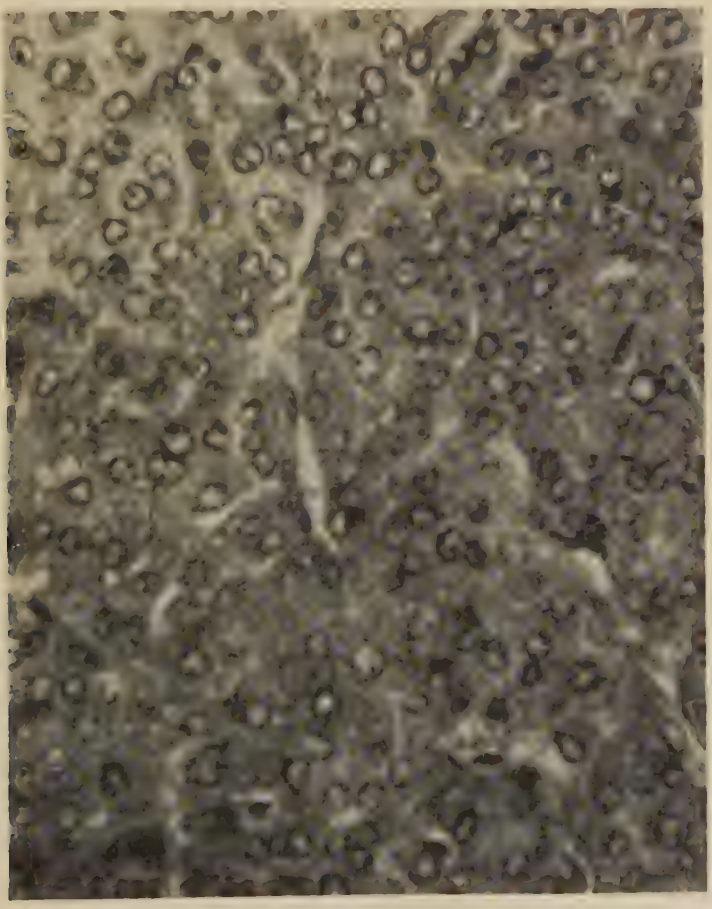


FIG. 2.
Chromophobe adenoma of the anterior lobe.

The cells contained no chromophil granules but were more or less uniformly basophilic and resembled the normal intermediate lobe cells in every respect except that they were considerably larger and somewhat less basophilic than the latter. The anterior lobe of the same hypophysis contained 2 small chromophobe adenomata of the type which is usually produced by prolonged oestrin treatment (Fig. 2).

Intermediate lobe tumors are exceedingly rare; in fact, outside of the small adenoma reported in our previously mentioned communication and the tumor described in this paper, to our knowledge no growth of this type has ever been reported. Among the many pituitaries of rats from our colony, we have never seen intermediate lobe tumors either in untreated animals or in rats belonging to other experimental series. Although we have no proof that the 2 intermediate lobe tumors which we observed are the result of oestrin treatment, we feel that, considering the great rarity of these tumors, they should be reported and emphasis should be laid on the fact that both of them occurred in oestrin-treated animals. It is also noteworthy that in the case here reported, the pituitary showed obvious signs of stimulation by oestrin inasmuch as two independent chromophobe adenomata such as are usually produced by oestrogenic substances were present in the anterior lobe.

*Summary.* A pigmented adenoma of the intermediate lobe of the hypophysis is described. This is the second time such a tumor has been observed and both cases were seen in rats chronically treated with oestrogenic preparations.

10369

## The Action of Toxic Doses of Atropine on the Central Nervous System.

THEODORE KOPPANYI.

*From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine.*

Scattered data concerning stimulation and depression of the central nervous system following the administration of atropine are both numerous and contradictory. Albertoni[1] reported that atropine in doses from 2-10 mg per kg increases the electrical excita-

[1] Albertoni, *Arch. Exp. Path. Pharmakol.*, 1882, **15**, 248.

bility of the motor cortex and facilitates the production of epileptiform seizures following stimulation of motor areas in dogs and monkeys, and stated that fatal or nearly fatal doses exert depressant or paralysant effects (terminating in paralysis of the limbs and respiratory muscles) without previous stimulation. Fraser[2] and Issekutz[3] described after initial depression the appearance of strychnine-like convulsions in frogs.

Joel,[4] Garcia,[5] Silver[6] and Friedberg[7] reported that the combination of atropine or hyoscyamine or scopolamine with morphine, ether, sodium phenobarbital or pernoston enhances the action of these narcotics. These authors used nearly anesthetic doses of the aliphatic depressants and supplemented these with doses from 25 to 50 mg of atropine per kg, immediately or a few minutes later. It is significant that Friedberg states that the synergism does not occur if the atropine is given one-half hour after injection of the barbiturates. All these experiments were carried out in rodents and it is obvious that a possible shock-producing effect of atropine given immediately on top of a sizeable dose of a central depressant, was not eliminated.

Very recently Schweitzer and Wright[8] have shown that 0.5-3.0 mg of atropine per kg depress the knee jerk owing to a central action and that these doses do not markedly antagonize either the excitatory action of physostigmine or the characteristic central inhibition produced by larger doses of acetylcholine.

The following experiments were carried out to study separately the stimulant and depressant actions of atropine and subject them to pharmacodynamic analysis. This was found possible only if large doses of this drug were employed.

A. *Frogs.* In *Rana pipiens* ("winterfrogs") intramuscularly administered doses of atropine sulfate from 50 to 400 mg per kg (20 animals) produced no observable effects; doses of 500 mg* (9 animals) produced sensory and motor depression lasting from 7 to 48 hours, following the depression 4 of the 9 animals showed hyperexcitability and an occasional convulsion upon sensory stimulation which persisted after destruction of the forebrain, became less pronounced after destroying the medulla and disappeared after pithing

2 Fraser, *Trans. Roy. Soc. Edinburgh,* 1869, **25**, 450.

3 Issekutz, *Z. Exp. Path. Therap.,* 1917, **19**, 99.

4 Joel, *Arch. Exp. Path. Pharmakol.,* 1928, **132**, 63.

5 Garcia, *Arch. Exp. Path. Pharmakol.,* 1928, **134**, 148.

6 Silver, *Arch. Exp. Path. Pharmakol.,* 1930, **158**, 219.

7 Friedberg, *Arch. Exp. Path. Pharmakol.,* 1931, **160**, 276.

8 Schweitzer and Wright, *J. Physiol.,* 1937, **89**, 384.

* All doses used are expressed in mg per kg of body weight; for the sake of brevity the last phrase is omitted.

the cord. Doses of 750 mg of atropine (5 animals) produced similar effects (2 of the 5 frogs developed hyperexcitability and spasticity), but 2 animals died within 6 days following administration. Doses of 1000 mg or more (10 animals) were invariably fatal, the sensory, motor and respiratory paralysis developing shortly after administration, persisting until death; no convulsions and in the majority of cases no curariform effects (judged by sciatic stimulation) were observed.

B. *Mammals.* Dogs, and to a lesser degree, cats are fairly tolerant to large doses of atropine. A preliminary survey of the toxicity of intravenously administered doses of this drug revealed that all but 2 dogs receiving 50 mg (12 animals) survived, 2 dogs receiving 60 mg survived, 4 of 5 animals receiving 75 mg recovered, while of those receiving 100 mg, 3 died and 3 survived. The drug seems to be more toxic for older than for younger animals. Atropine is far more toxic for cats, but doses from 30 to 40 mg are usually tolerated. The administration of these large doses of atropine is followed by a curious mixture of depression and stimulation. Immediately after injection depression predominates, the animals show muscular weakness, prostration, assume a lateral position and occasionally show relaxation and anesthesia of brief duration. They may fall asleep, but can be roused. It may take hours, but often only 15-30 minutes until the animals are able to stand or walk. A rapid fall of blood pressure following atropinization may be a contributing factor in the production of depression. In this series only dogs given 60 mg of atropine or more showed almost immediate typical epileptiform convulsions but occasionally dogs receiving the smaller doses exhibited other phenomena of central nervous stimulation: spontaneous elicitation of scratch reflex in rapid succession, stimulation of respiration, howling, retching and vomiting, hyperexcitability and spasticity. One dog, receiving 40 mg developed 2 hours after atropine administration clonic spasms. All these signs of stimulation, including the convulsions with higher doses are immediately stopped by small doses of barbiturates (*e. g.,* 10-15 mg of nembutal). The convulsions are not asphyxial; they continue in spite of vigorous artificial respiration and often cause death.

The following experiments were devised to ascertain whether the depression and stimulation are primary effects of atropine on the central nervous system or are secondary to drug injury of other structures.

Seven dogs each receiving 50 mg and 1 cat receiving 30 mg of atropine sulfate showed only a brief depression and within 30 min-

utes were able to stand and walk. Each of these animals received about one hour later 15 mg nembutal by vein and almost immediately following injection showed complete muscular relaxation and "surgical" anesthesia, (corresponding to an effect produced by 35-40 mg in control animals) recovering in 3½ to 4½ hours. On each of 4 successive days the same animals were again given 15 mg of nembutal and showed relaxation and anesthesia of gradually diminishing durations until on the fourth or fifth day following the administration of atropine they showed only motor incoördination and somnolence, *i. e.*, an effect 15 mg of nembutal usually produces in an untreated animal. One dog died on the next day following atropinization after the injection of 15 mg of nembutal. Two dogs receiving 50 mg of homatropine showed similar synergism with 15 mg of nembutal, but showed a normal reaction to the barbiturate on the third day. Similar results were obtained in 6 additional dogs by using 50-75 mg of sodium barbital instead of nembutal.

A typical condensed protocol may serve as an illustration:

| | | |
|---|---|---|
| Dog 9.2 kg; male | | November 9, 1938 |
| 10:35 | A.M. | 50 mg of atropine $SO_4$ by vein |
| 10:37 | " | Animal falls, defecates, howls |
| 11:00 | " | Able to stand |
| 11:15 | " | 15 mg nembutal, vein; falls |
| 11:16 | " | Anesthesia, relaxation |
| 12:18 | P.M. | Running movements, shivering |
| 1:10 | " | Attempts to right itself; howls |
| 2:20 | " | Crawls about |
| 4:10 | " | Able to stand |
| | | November 10, 1938 |
| 10:00 | A.M. | Animal appears normal |
| 10:35 | " | 15 mg nembutal; falls |
| 10:36 | " | Anesthesia, relaxation |
| 11:28 | " | Shivering, running movements |
| 12:00 | P.M. | Attempts to right itself |
| 1:35 | " | Recovery (able to stand without support) |
| | | November 11, 1938 |
| 9:45 | A.M. | Animal normal |
| 10:10 | " | 15 mg nembutal, falls |
| 10:20 | " | Anesthesia, relaxation |
| 10:50 | " | Shivering |
| 11:20 | " | Attempts to sit up |
| 12:18 | P.M. | Recovery |
| | | November 13, 1938 |
| 10:12 | A.M. | 15 mg nembutal |
| 10:15 | " | Lies down, holds up his head (no anesthesia or relaxation) |
| 10:21 | " | Sits up |
| 12:00 | M. | Complete recovery |

Doses of atropine less than 20 mg or of nembutal less than 10 mg cannot be used to demonstrate the above described phenomenon; by using 20-25 mg of atropine it is often possible to show severe depression following 15 mg of nembutal on the first day, but even then there is no anesthesia or complete relaxation. Smaller doses of nembutal (5-10 mg) do not produce complete relaxation and anesthesia irrespective of the dose of atropine.

Six dogs received 0.075 mg of strychnine sulfate (by vein); to 3 additional dogs the same dose was given twice within one hour without producing convulsions. To 5 dogs a dose of 50 mg of atropine was given which produced motor depression ("weakness of the limbs") and somnolence. Each of these 5 dogs received in about 30 minutes a dose of 0.075 mg of strychnine. Every one of these animals stood up almost immediately following injection, showed motor excitement and developed within a few minutes typical strychnine convulsions. Two of these 5 dogs died of convulsions. The surviving animals were given the same dose of strychnine the following day, but no convulsions were noted.

These experiments prove that (a) atropine, until appreciable elimination occurs, shows synergism with an aliphatic hypnotic; (b) large doses of the same drug synergize with a spinal convulsant, and (c) hypnotics may antagonize atropine stimulation and convulsants may oppose atropine depression. It other words, atropine, like morphine, has a twofold action on the central nervous system. Whether or not these actions can be explained by the acetylcholine-blocking effect of atropine still remains to be determined.

**10370**

## Anaphylaxis in the Pregnant Rat.

OSCAR D. RATNOFF. (Introduced by F. P. Gay.)

*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.*

Attempts have been made to elucidate the rôle of the endocrine glands in determining the susceptibility of animals to anaphylactic shock. Kepinow and Lanzenberg[1] found that thyroidectomy protected the guinea pig and rabbit against anaphylactic shock by in-

[1] Kepinow, L., and Lanzenberg, A., *Compt. rend. Soc. de Biol.*, 1922, **86**, 204, 906.

hibiting active sensitization with antigen. This, however, could not be repeated by Applemans[2] nor Yun;[3] and Fleisher and Wilhelmj[4] thought the protection was much less complete than the former authors believed. Blom[5] demonstrated the converse, that injection of thyroid preparations to sensitized guinea-pigs during the period of sensitization or shortly before the shocking dose, markedly increased the severity of anaphylactic shock; his method of injecting the shocking dose of antigen directly into the heart is, however, open to question.

Adrenalectomy was shown by Kepinow,[6] in guinea pigs, and Flashman,[7] and Wyman,[8] in rats, to increase the susceptibility of these animals to anaphylactic shock. Epinephrin has long been known to protect against the symptoms of anaphylaxis.[9] Wolfram and Zwemer[10] showed that cortin administered from 2 to 6 hours before a shocking dose protected a large proportion of guinea pigs against anaphylaxis; and Dragstedt, Mills and Mead[11] noted that cortical extract administered to dogs 48 hours to 5 minutes before diminished the severity of, but did not prevent anaphylactic shock.

Administration of parathyroid extract has led to contradictory results. Levinson and Matthews[12] claimed that amounts sufficient to raise the blood calcium of dogs to 15 mg % was without influence on the frequency of anaphylactic shock. Hajós,[13] on the other hand, found that the injection of parathyroid extract (Collip) decreased the sensitivity of guinea pigs to anaphylaxis.

Hajós also reported that, while anterior pituitary extracts were without influence on anaphylaxis in the guinea pig, posterior lobe extract caused a decrease in sensitivity. Cruveilheir and his associates,[14] however, found that the gonadotropic hormone adminis-

2 Applemans, R., *Compt. rend. Soc. de Biol.*, 1922, **87**, 1242.

3 Yun, *Zentralbl. f. d. ges. Hyg.*, 1930, **21**, 245, quoted by Blom.

4 Fleisher, M. S., and Wilhelmj, C. M., *Z. f. Immunität.*, 1927, **51**, 115.

5 Blom, T., *Z. f. Immunität.*, 1934, **83**, 373.

6 Kepinow, L., *Compt. rend. Soc. de Biol.*, 1922, **87**, 327.

7 Flashman, D. H., *J. Infect. Dis.*, 1926, **38**, 461.

8 Wyman, L. C., *Am. J. Physiol.*, 1929, **89**, 356.

9 Anderson, J. F., and Schultz, W. H., PROC. SOC. EXP. BIOL. AND MED., 1909, **7**, 32.

10 Wolfram, J., and Zwemer, R. L., *J. Exp. Med.*, 1935, **61**, 9.

11 Dragstedt, C., Mills, M., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1937, **59**, 359.

12 Levinson, S. A., and Matthews, S. A., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 350.

13 Hajós, C., *Endocrinology*, 1926, **10**, 560.

14 Cruveilhier, L., Haguenau, J., Thieulin, G., and Viala, C., *Compt. rend. Soc. de Biol.*, 1938, **128**, 282.

tered prior to injection of the shocking dose of antigen decreased their susceptibility to anaphylaxis. This protection Solomonica and Kurzrok[15] showed to be true likewise for anterior pituitary extract and for pregnancy urine. Molomut[16] conversely found that following total hypophysectomy via the pharyngeal route susceptibility to anaphylaxis in the ordinarily refractory rat was markedly increased, despite the animals' general hypometabolism.

Hajós[10] administered ovarian, corpus luteum, and male sex hormone extracts to guinea pigs without effect on anaphylaxis. Solomonica and Kurzrok,[15] however, found that follicular hormone conferred protection to guinea pigs. Schafer[17] could not confirm this, and believed guinea pigs given large doses of follicular hormone had a slightly greater susceptibility to anaphylaxis than his control group.

The influence of pregnancy on anaphylaxis is not clear. Jackson[18] noted that of 8 animals who died of anaphylactic shock in a series of 10 female and 19 male rabbits, 6 were females. All of these females were either pregnant or "pseudo-pregnant," *i. e.*, histological examination of the uterus showed the endometrium to be under the influence of corpus luteum hormone, although no embryos were present. However, 3 of the 4 female rabbits who survived injection of a shocking dose of antigen were likewise pregnant; the precipitin titers of these animals were uniformly below the level found in all rabbits who died in anaphylactic shock. Those females who shocked fatally had all survived injection of a shocking dose of antigen while in a non-pregnant condition, although 5 of the 6 had precipitin titers as high as those present in animals dying in anaphylaxis.

A study was made of the effect of pregnancy on anaphylaxis in the rat. This animal was chosen because of its natural refractoriness to anaphylaxis, rendering any increased susceptibility to shock more readily apparent. Twelve stock female rats of the hooded strain, obtained from Dr. P. E. Smith, were mated with stock breeder males. The onset of pregnancy was dated from the time of appearance of spermatozoa in the vaginal smear. As soon as impregnated, the rats were segregated from the males; within 24-36 hours after the time of impregnation, 1 cc of normal horse serum was injected intravenously. This injection was repeated 3 and 6 days later. Eleven days after the last injection, a "shocking" dose of 1 cc of the same horse serum was injected intravenously. The progress of the animal was noted by clinical observation, and in 9 of the rats

[15] Solomonica, B., and Kurzrok, R., *Endocrinology*, 1936, **20**, 171.

[16] Personal communication.

[17] Schafer, W., *Zentralblatt f. Bakt.*, 1938, **140**, 260.

[18] Jackson, C., *J. Immunology*, 1935, **28**, 225.

by following the rectal temperatures. Results were compared with 17 normal nonpregnant female control rats of the same stock, sensitized and shocked in identical manner.

Table I tabulates the degree of shock in the pregnant and in the control animals. It can be seen that there is no significant difference between the frequency of anaphylactic shock in the pregnant and in the nonpregnant animals. Two severe shocks occurred in the pregnant group, and 4 in the nonpregnant, but no animals died of shock in either series. Impregnation was corroborated by either postmortem examination or the subsequent birth of young. The experience of sensitization and shock had no demonstrable effect on the number of rats in each litter.

It can be concluded that in the rat the endocrine rearrangements of pregnancy have no bearing on susceptibility to anaphylaxis.

TABLE I.

A. Anaphylaxis in Rats 18 Days Pregnant at the Time of Injection of the Shocking Dose of Antigen (1 cc horse serum intravenously).

| Rat No. | Severity of Shock | Remarks |
|---|---|---|
| 111 | No shock | |
| 120 | " | |
| 109 | " | |
| 102 | Severe | Temp. dropped from 100.1 to 95.7°F |
| 104 | No shock | T. stationary |
| 108 | Mild | T. dropped to 96.7 |
| 115 | " | T. " from 101.7 to 98.4 |
| 100 | Severe | T. " " 101.7 to 96.8 |
| 110 | No shock | T. " " 99.6 to 98.4 |
| 105 | Mild | T. " " 98.5 to 96.0 |
| 114 | No shock | T. stationary |
| 122 | " | T. stationary; only .5 cc horse serum given intravenously |

B. Anaphylaxis in Control, Non-pregnant Female Rats.

| Rat No. | Severity of Shock |
|---|---|
| 37 | No shock |
| 38 | " |
| 39 | " |
| 40 | " |
| 41 | " |
| 42 | " |
| 43 | " |
| 64 | " |
| 61 | Severe |
| 62 | Mild |
| 63 | Severe |
| 65 | Mild |
| 66 | No shock |
| 67 | " |
| 68 | Severe |
| 69 | " |
| 70 | No shock |

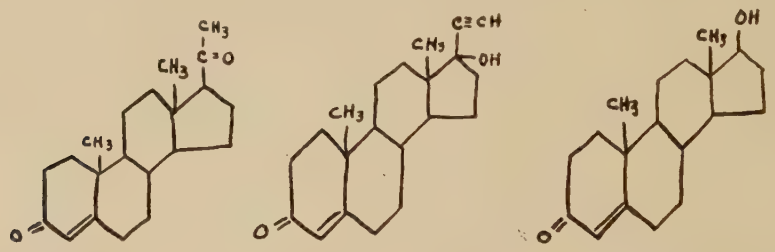
10371

# Production of Progestational Endometrium in Post-Menopause Women with $\Delta^4$ Pregnen-in-17-on-3-ol-17 Administered Orally.

Udall J. Salmon, Robert I. Walter* and Samuel H. Geist.

*From the Mt. Sinai Hospital, New York.*

Inhoffen and Hohlweg[1] have reported the production of progestational endometrium in primed, immature rabbits following the oral administration of $\Delta^4$ pregnen-in-17-on-3-ol-17† (pregneninolone, 17 ethenyl testosterone). This substance is a synthetic compound similar in structure to progesterone and testosterone.




Progesterone $\Delta^4$ Pregnen-in-17-on-3-ol-17 Testosterone

The present study was undertaken to determine whether this compound was effective in women when administered orally. The production of progestational changes in the endometrium of post-menopause women was used as objective evidence of progesterone-like activity. Five post-menopause women, in whom endometrial biopsies revealed advanced atrophy of the endometrium, were selected, primed with estradiol-benzoate† and then given pregneninolone by mouth in doses varying from 105 to 480 mg over periods varying from 3 to 8 days. Endometrial biopsies were obtained by suction curettage during the administration of the estradiol-benzoate as well as after the pregneninolone.

The following are protocols of 2 typical cases:

Case I. C.R.; age 44; natural menopause, 7 months. A preliminary suction curettage was attempted but, because of atrophy of the cervix with stenosis of the cervical canal, no endometrium was obtained. The patient was then given 350,000 R. U. of estradiol-benzoate, intramuscularly, in divided doses over a period of 10

* Joseph Brettauer Fellow in Gynecology.

† We are indebted to the Schering Corporation, Bloomfield, N. J., for the $\Delta^4$ pregnen-in-17-on-3-ol-17 and the estradiol-benzoate used in this investigation.

[1] Inhoffen, H. H., and Hohlweg, W., *Naturwissenschaften*, 1938, **26**, 96.

weeks. During the period of estrogen administration a suction curettage was performed and revealed a proliferative endometrium. The patient then received 140 mg of pregneninolone, orally, in divided doses, over a period of 4 days. Seven days after the beginning of the pregneninolone, another suction curettage was performed and revealed a definite progestational endometrium. These changes were characterized by: (a) nuclear shift in the glandular epithelium to the base; (b) subnuclear vacuolization; (c) tortuosity and serration of the glands; (d) edema of the stroma and increase in size of the stroma cells; (e) marked reduction in the number of mitotic figures. In many instances, glands in the proliferative stage were seen adjacent to glands exhibiting advanced progesterone effect.

Case II. E.V.; age 47; natural menopause, 2 years. A preliminary suction curettage revealed atrophic endometrium. The patient was then primed with a total of 655,000 R.U. of estradiol-benzoate over a period of 5 months. During this time 5 episodes of spontaneous bleeding, lasting from 1 to 5 days, occurred. Five endometrial biopsies were performed during the period of estrogen administration and revealed varying degrees of proliferation (Fig. 1). The

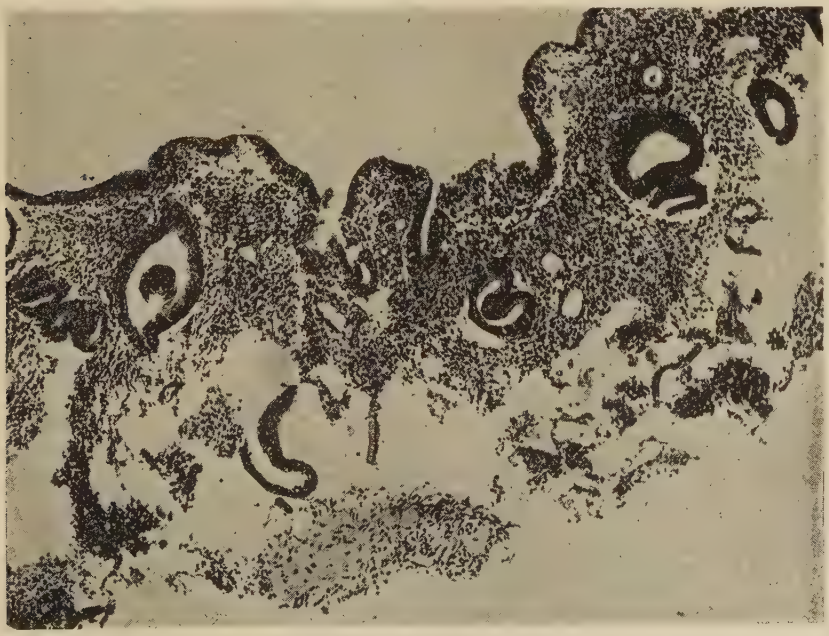


FIG. 1.

Case II. E.V. Age 47; natural menopause 2 years ago. Endometrial biopsy after priming with estradiol benzoate showing proliferative endometrium.

patient then received 60 mg of pregneninolone daily over a period of 8 days (480 mg). During the period of pregneninolone administration, 120,000 R.U. of estradiol-benzoate was given, intramuscularly, in divided doses. A suction curettage, performed on the 8th day after the beginning of pregneninolone administration, revealed a characteristic progestational endometrium (Fig. 2). Most of the glandular epithelium had the typical morphologic characteristics of progestational endometrium. In addition, the stromal changes were well marked and in some areas strongly suggestive of an early decidual reaction.

Of the 3 remaining cases, 2 showed striking progestational changes after 500 and 540 mg of pregneninolone given over periods of 8 and 10 days, respectively. In the third case, receiving only 105 mg of pregneninolone over a period of 3 days, the progestational effect was slight in degree with occasional glands exhibiting typical progestational characteristics.

*Summary.* Definite progestational changes were produced in the endometrium of post-menopause women with $\Delta^4$ pregnen-in-17-on-3-ol-17, administered orally, after preliminary priming with estradiol-benzoate.

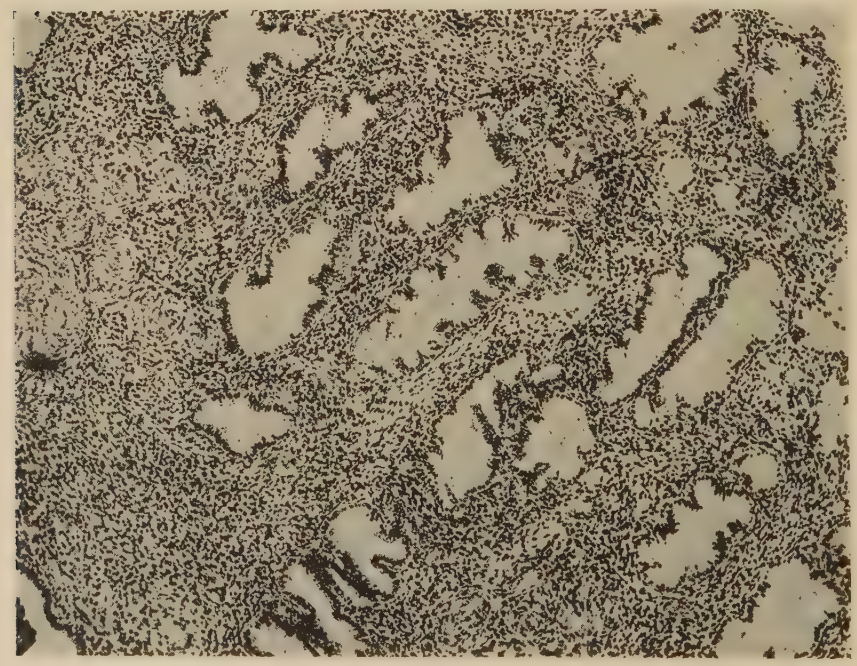


FIG. 2.
Endometrial biopsy performed 8 days after beginning of oral administration of pregneninolone. Total dosage 480 mg. Note typical progestational effect.

10372

## Effect of Oxygen Pre-Breathing on Anoxemia in Albino Rats.

Francis Marsh Baldwin and Harold D. Robinson.
*From the Department of Zoology, University of Southern California.*

While engaged in the study of a problem involving the changing erythrocyte counts in the blood of albino rats under low oxygen tensions,[1] it was noted that certain animals withstood the onset of critical anoxemia better than others. This observation, coupled with a report by Hill[2] concerning beneficial effects of oxygen pre-breathing upon aviators, stimulated the present inquiry as to (1) what effect intervals of oxygen pre-breathing might have upon the postponement of critical anoxemia in male and female groups of rats, and (2) what intervals might prove to be most advantageous, if effective at all, for these forms.

This report includes an analysis of the data taken from 4 series of experiments on 5 male and 5 female Wistar strain rats of comparable ages and weights selected from the department colony. Preliminary to the tests here cited, a number of experiments were performed in perfecting apparatus and technics. Each animal was subjected to intervals of pre-breathing of 25, 40, 70, and 130 minutes in a properly arranged chamber which could be exhausted and maintained at any given atmospheric pressure. As a control the same individuals were tested in air. The barometric pressure used was 200 mm Hg, which is equivalent to a partial oxygen pressure of only 42 mm Hg, or the approximate equivalent in altitude of 6½ miles above sea-level. The comparative differences in the time of onset of anoxemia in the different individual rats subjected to various intervals are shown in Table I. It is apparent from the analyses of these data that there are consistently slight differences in the 2 sexes, the females being slightly more resistant as a group than the males, when exposed to critical but not fatal low pressures (Table I). In fatal low pressures the females die in a shorter interval of time if no pre-breathing interval is administered.

When the data are resolved to averages and the pre-breathing intervals are plotted against resistance intervals in both sex groups, it is obvious that the 70-minute exposure is the most advantageous for both sexes. This is an interesting observation, since it appar-

1 Escobar, R. H., and Baldwin, F. M., *Am. J. Physiol.*, 1934, **107**, 249.
2 Hill, Leonard, *Nature*, London, 1932, **130**, 397.

TABLE I.

Critical Anoxemia in White Rats Following Varying Intervals of Pre-Breathing When Subjected to Low Oxygen Tensions (200 mm Hg.)

Pre-breathing intervals in minutes.

| No. | Sex | Air 25 min | Oxygen 25 min | Oxygen 40 min | Oxygen 70 min | Oxygen 130 min |
|---|---|---|---|---|---|---|
| | | min | min | min | min | min |
| 2 | ♂ | 11 | 20 | 14 | 26 | 15 |
| 8 | ♂ | 13 | 27 | 28 | 52 | 28 |
| 9 | ♂ | 12 | 36 | 25 | 31 | 30 |
| 10 | ♂ | 9 | 13 | 18 | 23 | 17 |
| 11 | ♂ | 13 | 26 | 23 | 42 | 22 |
| 3 | ♀ | 14 | 45 | 30 | 39 | 20 |
| 4 | ♀ | 10 | 29 | 35 | 44 | 26 |
| 5 | ♀ | 13 | 38 | 32 | 45 | 18 |
| 6 | ♀ | 19 | 33 | 40 | 32 | 39 |
| 7 | ♀ | 10 | 19 | 19 | 20 | 23 |
| Avg for all | | 12 | 29 | 26 | 35 | 24 |
| Avg for ♀ | | 13 | 33 | 31 | 36 | 25 |
| Avg for ♂ | | 12 | 24 | 22 | 35 | 22 |

Figures in the vertical columns are averages taken from 4 observations on each individual rat after exposures as indicated.

ently establishes a favorable optimum pre-breathing interval for rats. Why a shorter interval is better than the longer period of pre-breathing is not clear, but it would seem that toxic effects to longer exposures of pure oxygen result here, as were noted by Barach[3] on rabbits, and Haldane, Meakins and Priestley,[4] and Haldane, Kellas and Kennaway[5] on man.

In summary, it may be concluded that: (1) White rats as a class are normally quite resistant to low barometric pressures, but individual sex differences are apparent, the females being slightly more resistant than the males. 2. Pre-breathing oxygen for an interval of 25 or of 70 minutes is more advantageous in increasing resistance to anoxemia in both sexes, than no pre-breathing or pre-breathing for a longer interval. (3) The optimum pre-breathing interval from these data is 70 minutes.

[3] Barach, A. L., *Am. Rev. Tuber.*, 1926, **13**, 293.

[4] Haldane, J. S., Meakins, J. C., and Priestley, J. G., *J. Physiol.*, 1919, **52**, 431.

[5] Haldane, J. S., Kellas, A. M., and Kennaway, E. L., *J. Physiol.*, 1919, **53**, 205.

10373

## Wheat Germ Oil and Tumor Formation.

C. R. Halter. (Introduced by James Ewing.)
*From Memorial Hospital, New York City.*

Rowntree,[1, 2] and Dorrance and Ciccone[3] have reported the occurrence of tumors in rats fed wheat germ oil as an adjunct to their regular diet. It was spread on their daily rations in 1 to 4 cc amounts, or administered directly by mouth from pipette. As early as the 60th day of the experiment tumors (sarcomas) were observed in the abdominal cavities of the animals. They were transplanted successfully through as many as 15 successive recipients. It was determined that only ether-extracted oil was potent, and that the flocculent precipitate in the oil, rather than the oil itself was the effective principle.

Rowntree's methods of extracting the oil were considered too hazardous for us to pursue in a hospital caring for scores of patients. Following the suggestion of Dr. Fred R. Weedon (Dept. of Laboratories, Yonkers, N. Y.) we extracted our oil by ether direct, filtered it, and then cleared it of its ether by evaporation, first in an open room and finally in an incubator. Over half the extract was composed of a cloudy precipitate—called "foots" by the oil chemist.

In our first experiment 12 three-month-old rats of Wistar stock were given 1 cc doses of this "foots"-laden oil daily, directly by mouth from a pipette. Twelve control animals were fed "Zygon"—Squibb, an expressed wheat germ oil. Still continued 12 months after its inception this experiment is entirely negative for tumor production in our animals. In our colony of over 500 stock rats, living during this period, only one developed a sarcoma occurring in the abdominal cavity.

In the second experiment 7 rats, 4 to 7 months old, were given intraperitoneal injections of the oil in 1 cc doses, every 10 days, until each had received 10 such injections. Twelve months have elapsed since the beginning of this experiment. These animals are likewise free from tumors of any sort.

In the third experiment 4 rats, 5 months of age, were fed a very

1 Rowntree, L. G., Lansbury, J., and Steinberg, Arthur, Proc. Soc. Exp. Biol. and Med., 1937, **36**, 424.

2 Rowntree, L. G., *Am. J. Ca.*, 1937, **31**, 359.

3 Dorrance, G. M., and Ciccone, E. F., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 426.

highly concentrated wheat germ oil precipitate, extracted and generously donated by Mr. Reginald Auchincloss, of New York City. It was obtained from the ether-extracted oil by first mixing it with a 10% solution of NaOH in methanol, refluxing it 1½ hours, and then saponifying it. This was then mixed with an equal amount of water, extracted with ether 3 times, and washed in water 3 times. The extract was then dried over $Na_2SO_4$ over night and the ether finally distilled off leaving the unsaponifiable extract containing sterols and vitamin E. This product was so heavy it had to be liquefied by warming in giving it to the rats. Being about a 10-fold concentration of the supposed active principle only 2-minum doses were given the animals on alternate days. One of the rats died after it received 24 feedings, while the remaining 3, after having received 94 feedings, are still alive 4 months later. No tumors are discernible by palpation in these animals.

Although additional experiments are being continued and widened, the present series, now being concluded, admits of this report on our failure to produce tumors in rats through the administration of wheat germ oil.

**10374**

## Experimental Hypertension. Effects of Kieselguhr Injection and of Splanchnic Stimulation.*

RALPH D. CRESSMAN AND ALFRED BLALOCK.

*From the Department of Surgery, Vanderbilt University, Nashville.*

Several observers have determined the effects on the blood pressure of injecting multiple emboli into the renal arteries. Senator[1] injected liquid paraffin into the renal arteries of cats and no elevation of blood pressure occurred. Cash[2] injected insoluble Berlin blue into the renal arteries of dogs. The blood pressure did not become elevated. Similarly, no rise in the blood pressure of dogs was detected by Apfelbach and Jensen[3] following the injection of particles of charcoal. On the other hand, Maegraith and McLean[4] reported

* Aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

1 Senator, H., *Z. f. klin. med.*, 1911, **72**, 189.

2 Cash, J. R., *Bull. Johns Hopkins Hosp.*, 1924, **35**, 168.

3 Apfelbach, C. W., and Jensen, C. R., *J. Clin. Invest.*, 1931, **10**, 162.

4 Maegraith, B. G., and McLean, F. J., *J. Physiol.*, 1938, **92**, 44.

recently the results of experiments in which arterial hypertension was produced in rabbits by the injection of a suspension of Kieselguhr white into one renal artery. Even more interesting is their finding that the blood pressure returned to normal following the excision of the renal nerves. Renal denervation, or in fact total sympathectomy,[5] does not abolish in dogs the hypertension that is associated with partial constriction of the renal arteries by Goldblatt clamps. Because of this difference, the effect on the blood pressure of dogs of injecting Kieselguhr into the renal arteries has been determined.

A total of 14 dogs were used. Eight died after intervals of one to 21 days following the introduction into the right renal artery of from 0.5 to 20 cc of 1% suspension of Kieselguhr white. None of these animals developed a rise in blood pressure as determined by needle puncture of the femoral artery. Varying degrees of destruction of the kidney substance were observed. Complete liquefaction followed the introduction of the large doses. The right kidneys of 6 dogs surviving for 20 to 35 days the injection of the right renal arteries with from 1.0 to 6.5 cc of Kieselguhr suspension were explored and changes varying from complete necrosis to a small hard kidney were found. The left renal arteries of these dogs were then injected with from 0.25 to 2 cc of Kieselguhr. Three of these died in less than 7 days and moderate to severe infarction of renal tissue was found. On 3 subsequent occasions at intervals of 2 weeks, 0.5 cc of Kieselguhr suspension was introduced into one of the renal arteries of the 3 surviving dogs. An elevation in the blood pressure did not take place in any of these animals. Death followed the last injection and examination revealed hemorrhagic and anemic infarcted areas with marked degeneration.

The hypertension associated with the application of a Goldblatt clamp is believed to be due to an ischemia of living renal tissue. It is likely that Kieselguhr plugs the small renal vessels and results in a complete anemia with death of the part which is affected.

An entirely different type of experiment was performed on 3 dogs. Stimulation of the splanchnic nerves produces a vasoconstriction in the splanchnic area and a rise in blood pressure. To determine if prolonged mild stimulation of the splanchnic nerve in the unanesthetized dog would result in a sustained hypertension, a method similar to that described by Manning and Hall[6] was used. Unipolar electrodes were applied to the splanchnic nerve between the dia-

[5] Freeman, N. E., and Page, I., *Am. Heart J.*, 1937, **14**, 405.

[6] Manning, G. W., and Hall, G. E., *J. Lab. and Clin. Med.*, 1937, **23**, 306.

phragm and the upper pole of the adrenal gland. Three satisfactory preparations were obtained, 2 unilateral and one bilateral, in which the electrode did not pull out or cut through as determined by post-mortem examination in each case. Stimulation consisted of a 60 cycle alternating current of low voltage, from 0.1 to 0.5 volt, the voltage being raised to the point where the animal was annoyed by the stimulus, but below the level of pain as judged by reactions. The stimulus was alternately applied for 3 minutes and interrupted for 30 seconds in an attempt to avoid nerve fatigue. Except for a few minutes each day required to determine the blood pressure by the needle puncture method, the stimulus was maintained continuously for 17, 11, and 4 days in the 3 dogs. Except for one dog which showed a rise from a preoperative mean blood pressure of 115 mm Hg to an average of 155 mm Hg for the first 4 days, followed by a return to normal, no continued rise in pressure was demonstrated.

## 10375 P

## Development of Cardio-pericardial Adhesions Following the Use of Talc.

SAMUEL ALCOTT THOMPSON. (Introduced by I. S. Kleiner.)

*From the Surgical Department of the New York Medical College, Flower and Fifth Avenue Hospitals.*

Hydrous magnesium silicate (talc) powder was used inside the pericardial sac to produce adhesions of the pericardium to the epicardium. The reaction consisted in the development of a bread and butter pericarditis without the formation of fluid.

Adhesions occurred between the 2 surfaces as early as 18 hours. After one week the 2 surfaces were firmly adherent, and after 4 weeks the pericardium and the epicardium were fused as one layer of tissue.

There was no widespread development of adhesions beyond the area of the powder, the adhesions forming only at the site of the powder. The talc remained within the pericardial sac *in situ* and at autopsy was not discovered in the regional lymph glands.

The presence of new blood vessels between the pericardium and the epicardium was demonstrated at subsequent operations when bleeding was observed from both tissues when the 2 layers were separated. Also microscopic sections demonstrated the presence of new blood vessels.

In this series of experiments 46 operations were performed on 40 dogs. Under intraperitoneal nembutal anesthesia and intratracheal oxygen insufflation the chest was opened in the 5th interspace. The lung was manually compressed and held out of the way. The pericardium was opened and approximately one dram of the powder was lightly dropped and sprinkled onto the surface of the epicardium. The pericardium was then closed with a continuous suture. The lung was reëxpanded and the chest closed by percostal sutures and approximation of the soft tissues of the chest wall.

## 10376 P

## p-Bromophenol as the Intermediate in Synthesis of p-Bromophenylmercapturic Acid from Bromobenzene in the Rat.

JAKOB A. STEKOL AND HARRIMAN H. DASH.

*From the Department of Chemistry, Fordham University, New York.*

White and Lewis[1] in agreement with Coombs and Hele[2] demonstrated that the suggestion that in dogs "the oxidation to the phenol is a preliminary step in the synthesis of p-bromophenylmercapturic acid after the administration of bromobenzene appears improbable."[1] These workers reported that p-bromophenol, when fed to dogs, was in part excreted as an ethereal sulphate. No appreciable effect on the excretion of the neutral sulfur in the urine was noted. This observation indicated that apparently no p-bromophenylmercapturic acid was formed from p-bromophenol.

It appeared desirable to investigate the question of the synthesis of p-bromophenylmercapturic acid from p-bromophenol in the rat, using the direct method for the estimation of mercapturic acids in the urine. Aside from the fact that such an investigation had not previously been made on the rat, we felt that apparently the question of the formation of p-bromophenol from bromobenzene as a preliminary step in the synthesis of p-bromophenylmercapturic was not as yet settled. Thus Bodansky[3] in discussing the mechanism of the synthesis of the mercapturic acid from bromobenzene, states that "the reaction involves first oxidation to p-bromophenol and its subsequent conjugation with cysteine. The resulting p-bromophenyl-

1 White, A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **98**, 607.

2 Coombs, H. I., and Hele, T. S., *Biochem. J.*, 1927, **21**, 611.

3 Bodansky, M., *Introduction to Physiological Chemistry*, 4th edition, p. 393, 1938, John Wiley, New York.

TABLE I.

Effect of p-Bromophenol and Bromobenzene on Sulfur Partition and Excretion of p-Bromophenylmercapturic Acid in Urine of Rats.*

| Rat | Period | Total S mg | Inorganic $SO_4S$ mg | Ethereal $SO_4S$ mg | Neutral S mg | Mercapturic acid mg | Supplement with diet |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 24.50 | 16.11 | 3.47 | 4.92 | | None |
| | 2 | 23.29 | 9.23 | 9.65 | 4.41 | 0 | 210 mg p-bromophenol |
| | 3 | 93.04 | 75.56 | 12.48 | 5.00 | 0 | " " " and 420 mg $Na_2SO_4$ |
| | 4 | 31.34 | 19.62 | 6.22 | 5.50 | | None |
| | 5 | 36.47 | 6.26 | 5.20 | 25.01 | 197.3 | 210 mg bromobenzene |
| 2 | 1 | 26.34 | 16.57 | 4.00 | 5.77 | | None |
| | 2 | 25.60 | 10.21 | 8.77 | 6.42 | 0 | 210 mg p-bromophenol |
| | 3 | 76.72 | 57.86 | 11.08 | 7.78 | 0 | " " " and 420 mg $Na_2SO_4$ |
| | 4 | 29.16 | 17.20 | 3.97 | 7.99 | | None |
| | 5 | 39.57 | 11.95 | 4.28 | 23.34 | 152.8 | 210 mg bromobenzene |
| 3 | 1 | 31.84 | 13.71 | 4.88 | 16.25 | | None |
| | 2 | 29.15 | 8.72 | 8.44 | 12.99 | 0 | 210 mg p-bromophenol |
| | 3 | 103.84 | 78.02 | 12.30 | 13.52 | 0 | " " " and 420 mg $Na_2SO_4$ |
| | 4 | 32.32 | 16.54 | 5.82 | 9.96 | | None |
| | 5 | 43.51 | 12.38 | 4.34 | 26.79 | 160.0 | 210 mg bromobenzene |

*The results shown above are representative of experiments obtained with 8 rats. Each period in the table represents 6 days during which each rat ingested 42 g of diet C-10 with or without the supplements. The food was fed daily in 7 g portions and the urine was collected, under toluene, every 3 days. The pooled 6-day sample of urine was made up with water to a suitable volume and analyzed.

cysteine then undergoes acetylation." A similar statement is also made by Schmidt.[4]

We administered p-bromophenol to adult white rats which were fed constant amounts of a diet of uniform composition. The urine was collected and analyzed for total sulfur, inorganic and ethereal sulfates (Folin method) and for p-bromophenylmercapturic acid.[5] The urinary values were then compared with those obtained on the same rats which ingested the same amounts of the diet free from p-bromophenol. The diet C-10 had the following composition: casein 10, cane sugar 15, corn starch 46, yeast powder 5, Osborne-Mendel salt mixture[6] 4, cod liver oil 5, and Crisco 15. The supplements of p-bromophenol and $Na_2SO_4$ were fed mixed with the diet. The amounts of the supplements and the diet fed, the general procedure of collection of urine are indicated in Table I.

The results shown in Table I indicate that p-bromophenol, when fed to rats, causes an increased excretion of ethereal sulfates in the urine. The formation of the ethereal sulfates from p-bromophenol takes place apparently at the expense of the inorganic sulfates. No effect on the excretion of the neutral sulfur fraction of the urine is produced by the administration of p-bromophenol, nor any appreciable amount of p-bromophenylmercapturic acid is formed from this substance. On the other hand, comparable amounts of bromobenzene, when fed to the same rats under identical conditions, raised the output of the neutral sulfur in the urine which could be accounted for by the excretion of p-bromophenylmercapturic acid. The increase in the excretion of the ethereal sulfates sulfur during the p-bromophenol administration indicates that only 10 to 15% of the administered p-bromophenol is excreted as the ethereal sulfate. The major portion of the ingested p-bromophenol might, perhaps, be accounted for as a glucuronate. This possibility is being investigated.

The results are in complete accord with those of Coombs and Hele[2] and of White and Lewis,[1] and emphasize anew the improbability of the theory that p-bromophenol is the intermediate substance in the synthesis of p-bromophenylmercapturic acid from bromobenzene in dogs or rats.

Acknowledgement is made to the Works Progress Administration of the City of New York for the assistance rendered under project 665-97-3-39.

4 Schmidt, C. L. A., *The Chemistry of the Amino Acids and Proteins*, p. 236, 1938, Thomas, Springfield, Ill.

5 Stekol, J. A., *J. Biol. Chem.*, 1936, **113**, 279.

6 Osborne, T., and Mendel, L. B., *J. Biol. Chem.*, 1917, **37**, 572.

10377 P

## Hypertension from Obstruction of the Aorta.

ROBERT J. BROTCHNER. (Introduced by B. J. Clawson.)
*From the Department of Pathology, University of Minnesota.*

Goldblatt[1] and others have found in chronic experiments that by stenosing the renal arteries and thereby decreasing the blood supply to the kidneys a persistent elevation in blood pressure can be obtained. This phenomenon Goldblatt explains by the hypothesis of a "pressor substance" which he believes is formed as a result of the ischemia of the kidneys. He found also that stenosis of the aorta above the origin of both renal arteries resulted in a persistent elevation of blood pressure while stenosis below the origin of these vessels did not cause hypertension.

Rytand,[2] using heart weight increase in rats as evidence of hypertension, obtained similar results. Stenosis of the aorta below the right but above the left renal artery, he found, also produced hypertension; however, if the distal (left) kidney be removed hypertension does not result.

Goldblatt's[3] conclusion was that hypertension from experimental aortic stenosis is of the same nature as that due to stenosis of the renal arteries directly, that is, that the stenosis of the aorta caused an elevation of blood pressure by producing renal ischemia.

This idea was further expanded by Rytand to the supposition that renal ischemia with its resulting "pressor substance" is the important factor in the elevation of blood pressure so regularly found in the upper extremities in coarctation of the aorta.

Inasmuch as these results were obtained in chronic experiments it was felt that it would be interesting to investigate the aortic phenomenon with acute stenosis at similar levels.

Using dogs, intraperitoneal nembutal (pentabarbital) as anesthetic and carotid cannula recordings with the kymograph, the following experiments were performed:

1. A screw clamp was placed about the aorta just above the origin of the celiac axis, 2 or 3 cm above the origin of the right (upper) renal artery. The clamp was then closed at first partially

[1] Goldblatt, Harry, M.D., Experimental hypertension induced by renal ischemia, Harvey Lectures, 1937-1938.

[2] Rytand, David A., *J. Clin. Invest.*, 1938, **17**, 391.

[3] Goldblatt, Harry, M.D., *Transactions and Studies, College of Physicians of Philadelphia*, 1938, 4 Ser., Vol. 6, No. 1.

and then completely. Immediately after partial narrowing of the aorta the pressure began to rise and reached its maximum within several heartbeats. Further stenosis raised the pressure still more, the highest values being found with complete closure of the aorta and amounting to an average increase of 52 mm Hg in 4 experiments. This rise was maintained until the clamp was released. Upon release of the clamp there was an immediate drop in pressure, following which it gradually rose to about the original level. Moreover, partial release of the clamp caused a sudden but partial drop and the pressure readings could be decreased in steps by releasing the clamp a little at a time.

Next the renal arteries were ligated. No change in pressure was observed.

With the renal arteries ligated the aorta was again stenosed as above. Exactly the same type of rise and fall of blood pressure occurred. In one instance the rise was 76 mm Hg. This was maintained for over 40 minutes. Upon release of the clamp the same type of sudden drop occurred. The average rise in 2 experiments with the renal arteries ligatured was 65 mm Hg.

2. Stenosis and then complete closure of the aorta between the origins of the renal arteries was performed. The rise was rapid but small, averaging 13.5 mm Hg in 2 experiments. It was greatest with complete closure of the aorta. Release of the clamp gave a sudden drop with return to approximately the original level. With both renal arteries tied off a similar rise and fall were obtained.

3. Stenosis and complete closure of the aorta immediately below both kidneys caused a rapid but small rise of blood pressure with a sudden fall upon release of the clamp. With the renal arteries ligatured a similar result was obtained. The average rise in 7 experiments was 8.8 mm Hg.

TABLE I.

Percentage of Blood Flow Obstructed at the Different Levels and Average Rises of Blood Pressure Produced.

(A, liver and portal bed; B, kidneys; C, lower trunk and lower extremities.)

| Level of stenosis | % of flow obstructed (Levy, Blalock)[4] | Avg rise mm Hg | No. of exp. |
|---|---|---|---|
| 1. Above celiac | A + B + C = 60% | 56 | 6 |
| 2. Between kidneys | ½B + C = 22% | 16 | 3 |
| 3. Below kidneys | C = 13% | 8.8 | 7 |

[4] Levy, S. E., and Blalock, A., *Am. J. Physiol.*, 1937, **118**, 368.

10378

## Stimulation of Sensory Somatic Nerves in Relation to the Viscero-Pannicular Reflex.*

David M. Ashkenaz. (Introduced by J. C. Hinsey.)

*From the Department of Physiology, Cornell University Medical College, New York City.*

It has been demonstrated that the viscero-pannicular reflex (Ashkenaz and Spiegel[1]) is an objective, observable, constant criterion of the intactness of certain centripetal visceral pathways from the gall bladder of the cat. This reflex, which consists of a contraction of the panniculus carnosus muscle of the cat following adequate stimulation (distention) of a hollow viscus, such as the gall bladder, has been utilized in an analysis of centripetal conduction from the abdominal viscera of the cat (Ashkenaz[2]). Since this reflex is associated with the type of visceral stimulation (distention of a hollow viscus and stretching of smooth muscle) which ordinarily results in the sensation of pain in man, it has seemed interesting to determine whether stimulation of sensory nerves, other than visceral, known to conduct pain sensation, would also evoke a pannicular response.

The sciatic and phrenic nerves of decerebrate cats and cats under ether and dial anesthesia were stimulated by faradic stimuli from an inductorium as well as by repetitive stimulation with a thyratron stimulator but no indication of a contraction of the panniculus carnosus muscle was observed during such stimulation. If, during stimulation of the phrenic nerve, the splanchnic nerve were stimulated mechanically or the gall bladder were stretched or pulled, a contraction of the panniculus carnosus was observed, and this contraction ceased immediately upon cessation of the splanchnic or gall bladder stimulation, even though the phrenic stimulation were continued.

From these observations, it seems that a pannicular response is not usually and easily elicited by painful stimulation of afferent somatic nerves far removed from the segmental region supplying the panniculus carnosus.

*Summary.* Stimulation of the sciatic and phrenic nerves in de-

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

1 Ashkenaz, D. M., and Spiegel, E. A., *Am. J. Physiol.*, 1935, **112**, 573.

2 Ashkenaz, David M., *Ibid.*, 1937, **120**, 587.

cerebrate cats and in cats under ether and dial anesthesia by faradic stimulation with an inductorium or repetitive stimulation with a thyratron stimulator does not elicit a contraction of the panniculus carnosus muscle, but simultaneous or independent stimulation of the gall bladder or the splanchnic nerve (right) does evoke a contraction of the panniculus carnosus muscle.

## 10379 P

## Measurement of the Heart Size with the Roentgenkymograph.

ANCEL KEYS AND H. L. FRIEDELL.

*From the Departments of Physiology and Radiology, University of Minnesota Medical School, Minneapolis, Minn.*

The estimation of heart size in the living subject by the usual X-ray methods is generally inadequate in one or more respects: (1) the phase of the heart cycle is neglected—the total stroke output of the heart varies from 6% to 40% of the systolic heart volume, (2) it is assumed that simple linear measurements (*e.g.*, transverse diameter) accurately reflect true heart size (volume), (3) proper correction is not made for body size, (4) correction for distortion in the teleroentgenogram is disregarded, (5) accuracy is not proved by controls and duplicate measurement, (6) pendulum motion of the heart is included.

The roentgenkymograph, in which the film is moved during exposure behind a lead grid (Roesler;[1] Stumpf, Weber and Weltz;[2] Keys and Friedell[3]) registers the systolic and diastolic excursions of the heart contour, eliminates the effect of pendulum motion, and gives better visualization of the upper and lower borders.

We consider heart volume to be the ultimate reference measurement. Except with cadavers we have used frontal roentgenkymographs made at 66 to 72 inches with 2.5 second exposure, and have obtained the correction factors for distortion from lateral plates. All measurements were made on tracings of the X-ray plates and independently checked. Areas were measured with the planimeter.

1 Roesler, H., *Clinical Roentgenology of the Cardiovascular System,* Charles C. Thomas, Baltimore, Md., 1937.

2 Stumpf, P., Weber, H. H., and Weltz, G. A., *Röntgenkymographische Bewegungslehre innere Organe,* George Thieme, Leipzig, 1936.

3 Keys, A., and Friedell, H. L., *Science,* 1938, **88**, 456.

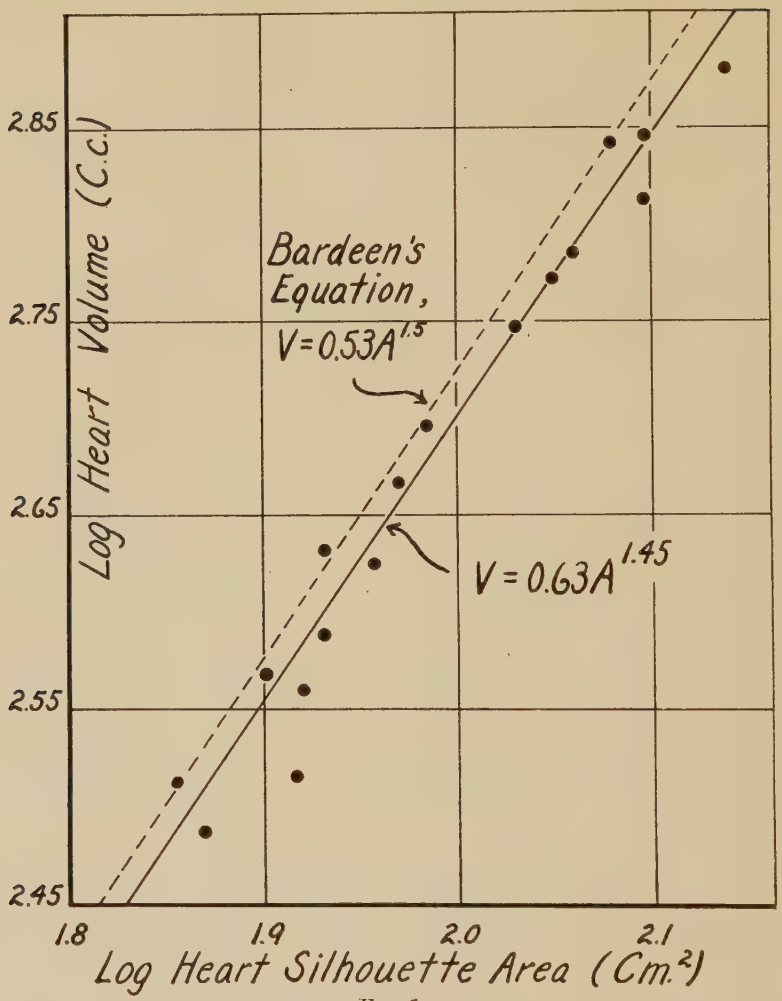



FIG. 1.

Relation between the corrected frontal silhouette area and the true total heart volume in 17 cadavers. Bardeen[4] assumed an exponent of 1.5; he included parts of the great vessels in the volume

Measurement of the frontal systolic silhouette area is very precise. Kymographs were repeated on 8 subjects at intervals of 2 to 10 minutes. The average deviation from the means for the 8 subjects was ±0.55% and the maximum deviation was only 1.6%. Three other subjects were studied from 4 to 6 times each over a period of 3 months. The average deviations from the means of the systolic areas for these subjects were ±0.7%, ±1.9%, and ±1.1%.

The accurate estimation of heart volume from a measurement of silhouette area would be possible if the heart may be considered to

be any combination of ellipsoids, spheroids or other regular geometric forms. The relation would be of the form:

$$\text{volume} = \text{constant} \times (\text{area})^{\text{exponent}}.$$

In a series of 17 cadavers we have made frontal teleroentgenograms and immediately thereafter we opened the chest, tied off the vessels and removed the heart for direct measurement. The relation in these hearts between the frontal silhouette area and the volume is shown

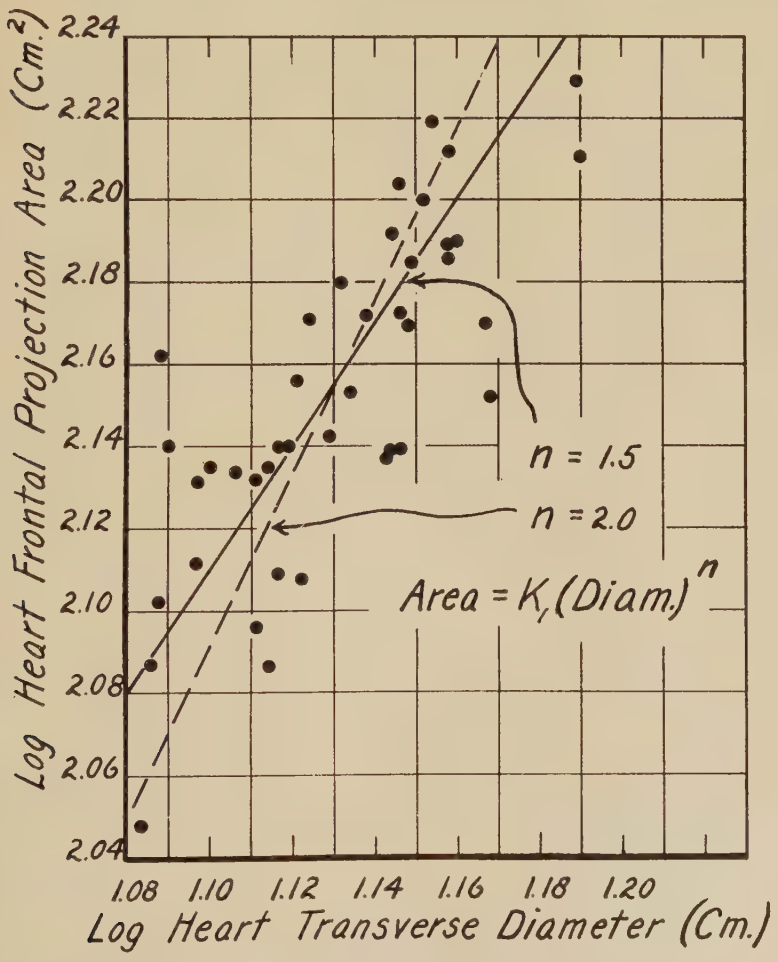



FIG. 2.

Relation between transverse diameter and the corrected frontal projection area in 41 normal cases. Measurements made from roentgenkymograph tracings, thereby eliminating questions of phase in the cardiac cycle, pendulum motion, distortion from divergent rays, etc.

in Fig. 1 together with the line of best fit and the line for Bardeen's[4] equation, which assumed perfect spheroidal form.

With our equation, $\text{Vol} = 0.63\ (\text{area})^{1.45}$, the average error in the estimation of heart volume from the frontal silhouette area was only ±4.4%; except for a single case where the error was —15.9%, the error was never greater than ±7%. For comparison we have made transverse diameter measurements on 41 kymograph plates from normal subjects. The relation between frontal t.d. to silhouette area is shown in Fig. 2. For this material the best prediction formula is: $\text{area} = 2.9(\text{t.d.})^{1.5}$, or $\log \text{area} = 1.5\ (\log \text{t.d.}) + 0.46$. The mean error in prediction of the area from the transverse diameter was ±4.9%, and the extremes were +14.2% and —15.0% of the true silhouette area. In pathological hearts the error is generally much larger.

The error in the estimation of heart size resulting from neglect of pendulum motion is variable but ±5% of the volume might be a fair average estimate for resting normal subjects. The mean errors from all sources may be summarized: (1) pendulum motion, +5%; (2) phase of the cardiac cycle, +20%; (3) irregularity in the shape of the frontal projection area (relation between area and transverse diameter), ±5%; (4) irregularity in the anterior-posterior shape of the heart (relation between frontal area and volume), ±4.5%; (5) neglect of distortion in teleroentgenograms, +12% (with a 6-foot plate). The largest errors from these sources may be estimated roughly: (1) +20%, (2) +40%, (3) ±15%, (4) ±15%, (5) +20%. Where frontal projection area is measured in teleroentgenograms inclusion of parts of the great vessels in the area may introduce a further error of the order of 5 to 10%.

When the roentgenkymograph is used as we do only error number 4 enters appreciably. We believe that this method makes possible accurate study of both functional and anatomical changes in given individuals. Precise comparison of different individuals is possible with this method provided adequate correction is made for body size. We believe this is accomplished by the expression of cardiac size in terms of units of total body surface as estimated from the usual height-weight formulæ (Keys and Friedell[8]). We have already established the relative constancy of the total systolic heart size per square meter of body surface in over 200 young men as measured by our method. Further work is in progress to extend these standards to females and to cover the entire age range.

[4] Bardeen, J. A., *Am. J. Anat.*, 1918, **23**, 423.

10380 P

## Value of Omentopexy in Establishing an Adventitious Circulation in the Normal Kidney.*

WILLIAM DEB. MACNIDER AND G. L. DONNELLY.

*From the Laboratory of Pharmacology, University of North Carolina.*

The following preliminary statement is based on studies which have been in progress at intervals for over 3 years. Fourteen normal dogs varying in age from one year and 2 months to animals in their sixth year have been used for experimental purposes. Under morphine-ether anesthesia the abdomen was entered through a left rectus muscle-splitting incision and the left kidney exposed. The kidney was delivered from its bed of perirenal fat, the capsule incised down to the cortical substance over the length of the kidney's outer convex border including the poles. The renal vessels were digitally compressed by an assistant. The cortex was then incised for distances in the respective kidneys varying from 3-8 cm, the incision extending through the cortex and into the medullary substance, but not sufficiently deep to penetrate into the pelvis of the kidney. Into such incisions the omentum was introduced and the cortical incision closed with fine, interrupted chromic cat gut sutures. Those sutures passing through the omentum were lightly tied in order to prevent compression of the omental vessels. The partially deflected capsule of the kidney was then brought up and sutured over the capsular incision. The abdominal incision was closed in the usual manner.

At intervals varying from 15 days to 3 months the animals were again anesthetized and 2 types of procedures followed. The omentum was either tied off from the renal cortex followed by the removal of an eliptically shaped mass of cortical and medullary tissue containing the omental implant or the entire kidney was removed. In the latter event one or more of the omental vessels were immediately injected with India ink. Following both types of procedure the renal tissue was fixed in Zenker's solution and imbedded in paraffin or celloidin. The paraffin impregnated tissue was used to obtain thin sections 3 to 8 micra, while the tissue imbedded in celloidin was employed for thicker sections arranged serially. Sections of both types were stained with hematoxylin and eosin and

* Aided by a grant from the fund for scientific research of the American Medical Association.

with Mallory's connective tissue stain. The India ink injected tissue greatly facilitates the study of the vascularization of the kidney by an ingrowth of capillaries from the omental implant.

The histological studies indicate that the earliest changes to develop from such an experimental procedure to consist in a disappearance of the omental fat which is in part replaced by an ingrowth of connective tissue cells and by cells of an endothelial order. The latter type of cell may contain fat droplets. The local cellular response of renal tissue to implantation of omental tissue is not characterized by a predominant small round cell invasion or by an infiltration of polynuclear cells. In such areas of cellular reaction there develops an ingrowth of capillary buds from the omental capillaries which are not confined to the area of the omental implant. Capillaries with an omental connection may be traced in serial sections, such tissue having been injected with India ink, in both a lateral direction into the renal cortical tissue and downward into the renal medulla for a distance beyond the location of the omental implant. In such medullary areas as was the case with the invasion of the cortex by the capillary ingrowth the vascularization takes a course lateral to the downward ingrowth of capillaries from the omental implant so that from such an implant a considerable area of renal tissue receives a blood supply from this adventitious source. In the cortex such capillary invasion has shown no tendency to emphasize its distribution around the glomerular tufts. The capillary network is most marked around the convoluted segments of the nephron. The question as to whether or not such capillary beds are solely dependent upon the ingrowth of such vessels from the omental implant or whether a part of the renal vascularization is due to an anastomosis of such capillaries with preëxisting capillaries of normal origin has not been determined. Associated with the ingrowth of capillary buds from the omental area into the cortical and medullary tissue which, in turn, is related to the duration of the respective experiments, there develops not only a hyperplasia of the cells of the walls of the primary omental vessels but an increase in the thickness of the walls of the capillaries which have initially invaded this tissue.

10381

## Uveal Tissue Sensitization in Rabbits by Synergic Action of Staphylotoxin.

HUGO LUCIC. (Introduced by E. L. Burky.)

*From the Wilmer Ophthalmological Institute, Johns Hopkins University and Hospital, Baltimore.*

Sympathetic ophthalmia is a disease of obscure etiology, characterized by a special type of inflammation of both eyes, involving chiefly the uveal tract, and usually follows a penetrating injury of one eye.

Although this disease is comparatively rare, its pathogenesis has offered fertile ground for speculation among ophthalmologists. Of the several theories proposed to explain its etiology, the so-called allergic theory is one of the most important. The purpose of this report is to summarize the approach to the problem of sensitization with uveal-tissue pigment in laboratory animals. A detailed report covering the methods used, the results obtained, and their relationship to experimental sympathetic ophthalmia will appear in an ophthalmological journal at a later date.

During the last 30 years a considerable amount of evidence has appeared in ophthalmic literature, indicating that hypersensitivity to uveal tissue pigment may play some part, as yet undetermined, in the pathogenesis of the disease. The so-called anaphylactic or allergic theory was first proposed by Elschnig, and it has been strongly supported, with an important qualification, by Woods[1] in this country. These and other authors have shown that uveal tissue possesses antigenic properties; that it can act as an antigen in the homologous animal; that it is organ-specific; and that it materially lacks species-specificity.

In the experimental approach to this problem, the difficulty has been in sensitizing laboratory animals with the insoluble uveal-tissue pigment. For instance, no one has been able to demonstrate in the laboratory animal a hypersensitivity to uveal pigment comparable to the reaction that occurs in the human in cases of sympathetic ophthalmia. This step, the production and demonstration of hypersensitivity to uveal pigment, is manifestly necessary before the relationship of pigment sensitivity to sympathetic ophthalmia can be evaluated experimentally.

---

[1] Woods, A. C., *Allergy and Immunity in Ophthalmology*, Baltimore, Johns Hopkins Press, 1933, pp. 67 ff.; *N. Y. State J. of Med.*, 1936, **36**, 1.

Burky[2] isolated a strain (Ha) of staphylococcus which produced an exotoxin toxic for adult rabbits. He discovered that by repeated intracutaneous injections of the toxin there was developed in the animal, not only an active immunity, but also a hypersensitive state to the broth in which the toxin had been produced. From this primary observation, Burky concluded that other substances with mild antigenic properties might become activated if combined with the toxin. By this method he succeeded in sensitizing rabbits to low-ragweed extract, lens-protein, and homologous muscle-tissue.

Following Burky's method, a "uveal-tissue-toxin" was prepared by aseptically removing uveal tissue from beef and rabbit eyes. This tissue was dropped into individual tubes containing hormone-bouillon. After a period of incubation, the contaminated tubes were discarded, and the sterile ones were inoculated with Burky's staphylococcus (Ha) and incubated for 2 weeks at 37°C. The contents of the tubes were pooled, enough trikresol was added to make a 0.5% solution and the material was then thoroughly macerated in a mortar and filtered through sterile gauze. An intravenous injection of this filtrate, consisting of 0.1 cc per kilo of body weight, given to an adult rabbit, killed the animal within 24 hours.

Fifty-three adult rabbits were used in the experiment. Twenty-two of these were used for bovine uveal-tissue-toxin sensitizing inoculations, 11 for rabbit uveal-tissue-toxin inoculations, and 20 served as controls for similar inoculations with uveal-tissue suspension without toxin. Preliminary to the sensitizing injections, all the rabbits were tested by an intracutaneous injection of a stock uveal-pigment suspension, and the injected skin was removed 2 weeks later for histologic examination as recommended by Friedenwald.[3] Sensitizing injections, consisting of 0.1 cc of the above preparations respectively, were given intracutaneously at intervals of one week for 4 weeks. Those animals that had received bovine uveal-tissue-toxin were tested with swine or rabbit uveal-pigment suspensions, and those which had received rabbit uveal-tissue-toxin were tested with swine uveal-tissue suspension. The control animals were tested with swine, rabbit, and autogenous uveal pigment. Six of the 22 animals that had received bovine uveal-tissue-toxin sensitizing inoculations proved hypersensitive at the end of this period. None of the 11 rabbits that had received homologous uveal-tissue-toxin proved hypersensitive. None of the 20 control animals showed a positive reaction.

---

[2] Burky, E. L., *J. Allergy*, 1934, **5**, 466.

[3] Friedenwald, J. S., *Am. J. Ophth.*, 1934, **17**, 1008.

To determine the reliability of the positive reaction, 2 of the hypersensitive animals were tested simultaneously with rabbit, swine, and autogenous uveal pigment. Both of these animals showed a uniformly positive histologic reaction to all 3 suspensions of pigment, thereby demonstrating once more the organ-specific antigenicity of uveal-tissue pigment. One of these animals was retested 8 months later and was found to be hypersensitive at the end of this period.

*Summary.* 1. A method of preparation of "uveal-tissue-toxin" is described. 2. Repeated intracutaneous injections with heterologous (bovine) uveal-tissue-toxin produced simultaneous sensitization to heterologous (swine), homologous, and autogenous uveal pigment in 6 out of 22 rabbits. 3. Similar sensitizing injections with homologous uveal-tissue-toxin produced no sensitization to uveal pigment in any of the 11 rabbits used. 4. Repeated injections of uveal-tissue devoid of staphylotoxin produced no sensitization to uveal pigment. 5. The criterion of hypersensitivity was based upon the histologic cellular reaction observed in tissues excised 2 weeks after the injection.

**10382 P**

## Cesium in the Mammalian Retina.*

GORDON H. SCOTT AND BRUCE L. CANAGA, JR.

*From the Department of Anatomy, Washington University School of Medicine, St. Louis.*

Fox and Ramage[1] and Sheldon and Ramage[2] reported that they were unable to identify, by spectrographic methods, cesium in the tissues of animals of widely divergent genera. Since it is well recognized that different methods of spectra excitation favor the identification of some elements it occurred to us that by use of the interrupted arc method (McMillen and Scott[3]) we might get the identifying lines. Fox and Ramage and Sheldon and Ramage used flame excited spectra in their studies. An examination of several hundred tissue and fluid samples from various mammals by our method failed to give any positive results.

* Aided by grant from the Josiah Macy, Jr., Foundation.

1 Fox and Ramage, *Proc. Roy. Soc. Lond.*, 1931, **108**, 157.

2 Sheldon and Ramage, *Biochem. J.*, 1931, **25**, 1608.

3 McMillen and Scott, *Rev. Sci. Inst.*, 1937, **8**, 121.

Through the kindness of Dr. Douglas Coles of The Veterinary Research Laboratories, Onderstepoort, Transvaal, South Africa, we obtained several ox eyes. In the course of routine spectrographic analysis of these specimens for barium, cesium was found in the retina. This led us to study spectrographically the retinae of some 60 eyes obtained from a local abattoir. Of this group 19 were from pigs, 17 from oxen and 24 were from sheep. Presumably the animals came from a rather wide though illy defined area of the Middle West. No more definite information could be obtained on this point.

By carefully controlling the method used it was possible for us to exclude contamination from other sources. The eyes were slit and fixed in cesium-free formaldehyde as it was found much easier to separate the retinal layers in the hardened material. The instruments used in manipulating the tissue did not add cesium to samples of other tissues and consequently it is not at all likely that they did so in this case. The tissues to be examined were allowed to digest in a small amount of cesium-free $HNO_3$ in chemically clean vessels. The resulting liquid was applied to cesium-free carbons and arced as described by McMillen and Scott (loc. cit.). A Bausch and Lomb medium Quartz Spectrograph with a slit width of 0.02 mm was used in our experiments. An exposure of 320 flashes of about 0.5 second duration was sufficient to produce good plates.

In our experiments we made a rough attempt to localize the element in the retina. The retinae were separated into 2 layers, the thinnest of these being the nerve fiber layer and internal limiting membrane and the thickest being the cellular layer which consisted of pigmented epithelium, rods and cones and their nuclei, neuroglial and bipolar cells. Cesium was identified without great trouble by the 4555.5 Å line and was checked repeatedly by comparisons with similar tissue to which the element had been deliberately added. In no case did we fail to recognize the element in both layers of the retina examined. There was no essential difference in the species or in the layers of the retinae in our series. It is evident from these findings that cesium may be presumed to exist constantly in the retinae of the species studied. Furthermore it is present in distinctly greater quantities than in other tissues and fluids of the body as in no case was it identified by similar or other methods except in the retina.

No attempt is made here to attach any physiological significance to the finding. We would suggest, however, that it is a peculiar coincidence that an element so active photoelectrically is found only in a tissue layer which is capable of translating photon impacts into nerve impulses.

10383

# Osmotic Pressure of Gum Acacia Solutions.

GEORGE SASLOW.

*From the Department of Physiology, Harvard School of Public Health, Boston, Mass., and the Marine Biological Laboratory, Woods Hole, Mass.*

Dodds and Haines[1] observed that the colloid osmotic pressure of sterilized 6% acacia solutions was much reduced by added salt, so that in the presence of 0.9% salt the pressure was about one-half that of the blood colloids of mammals. The addition of salt to acacia solutions was, therefore, considered to defeat the object sought in the intravenous administration of the gum,* and the problem becomes of moment in consideration of the use of acacia solutions as a plasma substitute both in physiology and in clinical medicine.

The successful use of acacia in total plasmapheresis experiments on dogs (Stanbury, Warweg and Amberson[2]), and some measurements of colloid osmotic pressure of acacia solutions used in perfusion experiments on frogs (Saslow[3]) suggested that the question be reinvestigated.

*Method and Materials.* The method used was that employed by Dodds and Haines,[1] namely, the second method of Krogh and Nakazawa.[4] The method is adequate to the purpose though admittedly far from ideal. The precision attained was of the same order as that reported by Wies and Peters,[5] namely, a standard deviation of $\pm 8$ mm $H_2O$ in a series of 20 determinations on one sample of 6% acacia made up in 0.9% NaCl (the colloid osmotic pressure of the sample at 20°C was 260 mm $H_2O$).

The membrane used was cellophane, plain, No. 600. During the course of the experiments the material was kept at 21°C and 30 to 40% relative humidity in the Precision Instrument room of the Marine Biological Laboratory. Before use, pieces of cellophane were soaked in the appropriate crystalloid solution for several hours.

1 Dodds, E. C., and Haines, R. T. M., *Biochem. J.*, 1934, **28**, 499.

* On the basis of their measurements, the object of intravenous administration of the gum would have been defeated by the salts present in blood, whether or not salt were added to the acacia before injection.

2 Stanbury, J., Warweg, E., and Amberson, W. R., *Am. J. Physiol.*, 1936, **117**, 230.

3 Saslow, G., *Am. J. Physiol.*, 1938, **124**, 360.

4 Krogh, A., and Nakazawa, F., *Biochem. Z.*, 1937, **188**, 241.

5 Wies, C. H., and Peters, J. P., *J. Clin. Invest.*, 1937, **16**, 93.

The cellophane was tested for acacia-tightness with an ultrafilter operated at 60 kg per square centimeter for 3 hours. To the ultrafiltrate was added a few drops of 3% lead subacetate (Ritsema[6]); a well marked precipitate is formed with acacia in concentrations down to 0.05%. The test is unreliable in dilutions of acacia greater than this. Test of numerous samples of the ultrafiltrate was negative, *i. e.*, less than 0.05% acacia is forced through the cellophane under the conditions described. If we take the osmotic pressure of acacia solutions as roughly linear with concentration (actually the pressure falls off at the low concentrations), the maximum error which could have occurred with supposed leakage of acacia of the order of 0.05% is 2 mm $H_2O$.

The acacia used for most of the measurements was part of a special lot, containing no added NaCl. This lot was prepared for us by the Eli Lilly Company from one large batch of gum and was used in perfusion experiments described recently (Saslow[3]). The mode of preparation of the acacia was as follows: The acacia used is described as "highest quality pearls, Grade No. 1"; the pearls were hand-selected for color, those with a dark tint being discarded. 1750 g of the selected acacia pearls were dissolved in 5 liters of water containing 25 g sodium glycerophosphate; the water was warmed to approximately 75°C to effect solution. The solution was autoclaved for 4 hours at 120°C, and then allowed to stand over night. A precipitate formed which was filtered off; the filtrate was adjusted to pH 6.4 to 6.7 with NaOH. The solution was heated on the water bath at 60°C for 15 minutes and then kept at 4° over night. The slight precipitate which formed was filtered off and the solution was diluted with sufficient freshly distilled water so that each 100 cc contained 27 to 30 g of solids. The diluted solution was filtered until clear and filled into ampoules. These were sealed and sterilized by boiling one-half hour on each of 3 successive days. The composition of this lot of acacia in "30% solution" was as follows: total solids, 26.8%; total ash, 1.0%; total base, 20.8 cc N/1 acid per 100 cc; Ca, 0.147%; K, 0.198%; Na, (by difference) 0.186%; Mg, 0.0025%; $PO_4$, less than 0.0004%; unidentified $H_2S$ metals as Cu, 0.025%. According to the experience of the Eli Lilly Company, the range of composition of different samples of 30% acacia similarly prepared is: Ca, 0.126 to 0.136%; Mg, 0.0015 to 0.0038%; pH, 5.0 to 5.2; $PO_4$, none; heavy metals, none by the tests in U.S.P. XI.

For purposes of comparison, the colloid osmotic pressure of sev-

[6] Ritsema, C., *Pharm. Weekblad.*, 1934, **72**, 105.

eral other lots of acacia solution was measured. These were prepared with added NaCl, by the Lilly Research Laboratories.

Measurements were made at temperatures of 21 to 25°C. In any one run, the variation in temperature was of the order of 1°. Final readings were taken from 20 to 24 hours after the osmometers had been set up. Trial showed that the colloid osmotic pressure became steady at about 16 hours and remained so for at least 70 hours. All values tabulated (Tables I and II) are the average of 6 to 10 individual measurements, and have been reduced to 20°.

*Results.* The colloid osmotic pressure of the special lot of acacia (referred to hereafter as Lot 1) in NaCl solutions of various concentrations is shown in Table I.

TABLE I.
Colloid Osmotic Pressure of 6% Acacia (Lot 1) in NaCl Solutions.

| | pH | | | Dodds and Haines[1] | |
|---|---|---|---|---|---|
| NaCl % | outside fluid | inside fluid | Colloid osmotic pressure mm $H_2O$ | Colloid osmotic pressure mm $H_2O$ | pH inside fluid |
| 0.00 | 5.58 | 5.60 | 773 | 841 | 4.5 |
| 0.05 | 5.63 | 5.51 | 555 | 531 | — |
| 0.10 | 5.50 | 5.47 | 462 | 350 | — |
| 0.15 | 5.60 | 5.43 | 415 | — | |
| 0.20 | 5.51 | 5.41 | 365 | 232 | — |
| 0.28 | | | — | 196 | 4.4 |
| 0.30 | 5.58 | 5.43 | 304 | — | |
| 0.31 | | | — | 193 | — |
| 0.46 | | | — | 170 | 4.3 |
| 0.50 | 5.49 | 5.38 | 285 | — | |
| 0.70 | 5.52 | 5.35 | 285 | — | |
| 0.90 | 5.50 | 5.32 | 260 | 128 | 4.2 |
| 1.00 | 5.49 | 5.31 | 238 | — | |
| 2.00 | | | — | 118 | — |

The colloid osmotic pressure of acacia Lot 1 was measured also in buffered Ringer-Locke's of the following composition: NaCl, 0.9%; KCl, 0.042%; $CaCl_2$, 0.024%; P($Na_2HPO_4$, $NaH_2PO_4$) 10 mg %; pH 7.20; $\mu = 0.176$; $\Delta = 0.699°$. The value obtained is compared in Table II with the colloid osmotic pressure of different lots of

TABLE II.
Colloid Osmotic Pressure of Different Lots of Acacia.

| No. of lot | Date sealed into ampoules | Date measured | Solution measured | Colloid O.P. mm $H_2O$ |
|---|---|---|---|---|
| 1 | 9/28/37 | 8/6/38 | 6% acacia in Ringer-Locke's | 243 |
| 1 | " | 6/23/38 | 6% " , 0.9% NaCl | 260 |
| 2 | 10/7/37 | 8/3/38 | 6% " , 0.9% " | 246 |
| 3 | 1/11/38 | 8/17/38 | 6% " , 0.9% " | 253 |
| 4 | 7/22/38 | 9/2/38 | 6% " , 0.9% " | 250 |

acacia prepared with added NaCl (the measurements were made on solutions diluted to 6% acacia, 0.9% NaCl).

The figures in Table II should be compared with some determinations of Wies and Peters,[5] who measured the colloid osmotic pressure of a sample of Lilly-prepared acacia with added NaCl and diluted to 6% acacia, 0.9% NaCl; the pressure at 20° was 252 mm $H_2O$. A similar value was obtained by Turner (quoted by Wies and Peters[5]) on another sample. Butt and Keys,[7] using a sample of acacia the origin and preparation of which are not stated, obtained the value 262 mm $H_2O$ for 6% acacia, 0.9% NaCl.

The colloid osmotic pressure of 6% acacia in 0.9% NaCl is approximately the same as the pressure of human serum proteins, as reported by Wies and Peters.[5] In their series of observations, the colloid osmotic pressure of 69 sera the serum protein concentrations of which lay between 6 and 8% covered the range 205 to 404 mm $H_2O$, with the average value at 276 mm $H_2O$.

The method used for the measurements described appears to give the same result as the much more laborious method (due to Adair) used by Butt and Keys[7] so far as the acacia available in the United States is concerned. Further, acacia prepared according to the procedure described seems to be a uniform and stable product, having in 6% solution in media approximating the salt content of blood about the same colloid osmotic pressure as serum itself.

That added salt lowers the colloid osmotic pressure of acacia solutions has been observed by several workers. A particularly complete study of the subject has been made by Oakley.[8] His experiments show that the osmotic pressure of acacia solutions depends greatly, as is to be expected of a polyvalent colloidal electrolyte, upon the qualitative and quantitative salt content of the medium, the pressure being greater the smaller the added salt content of the solution. As added salt is increased, the ionic pressure difference between colloid solution and dialysate decreases continuously. The precise form of the relation between the osmotic pressure and the added salt concentration will depend not only upon the amount and nature of the salt added but also upon the initial composition of the acacia solution itself. Should this have been so prepared as to have a relatively high total ash content, the osmotic pressure will fall more sharply and to lower levels upon the addition of given amounts of salt than if the original solution had a lower total ash content. An acacia solution

[7] Butt, H. F., and Keys, A. J., *Physical Chem.*, 1938, **42**, 21.

[8] Oakley, H. B., *Trans. Faraday Soc.*, 1934, **31**, 136; 1936, **32**, 1360; 1937, **33**, 372.

of lower initial pH will also show a lower osmotic pressure than one of higher pH (Oakley[8]). Since the composition of the acacia solutions used by Dodds and Haines[1] is not given in sufficient detail, it is impossible to say more than that very likely the explanation of the difference between their values for the colloid osmotic pressure of 6% acacia in 0.9% NaCl and the values of the various investigators cited here lies in differences in inorganic composition of the initial acacia solutions. Presumably the same explanation applies to the marked differences among the values reported for the colloid osmotic pressure of 6% acacia solutions by various investigators (quoted by Amberson[9] and Butt and Keys[7])—values ranging from 120 to 3740 mm $H_2O$; some of the differences arose, however, because measurements were made on solutions now of one salt content, now of another. Recent measurements made in the United States on 6% acacia in 0.9% NaCl or similar solutions seem to be in satisfactory agreement.

*Summary.* 1. The colloid osmotic pressure of several samples of 6% acacia in 0.9% NaCl has been determined to be 246 to 260 mm $H_2O$ at 20° C. 2. This value is approximately the same as the average colloid osmotic pressure of human sera ranging in protein concentration from 6 to 8%, namely, 276 mm $H_2O$. 3. Acacia solution processed by the procedure of the Lilly Research Laboratories appears to be a stable and uniform product.

**10384 P**

## Evidence of Another Factor in the B Complex for Rats.

W. R. WYATT. (Introduced by V. E. Nelson.)

*From the Laboratory of Physiological Chemistry, Iowa State College.*

$B_1$ (Thiamin), flavin, nicotinic acid, $B_6$, and the filtrate factor are generally accepted as distinct components of the B complex for rats. Recent work on other species of animals by Morgulis, Wilder, and Eppstein[1] and by Jukes and Babcock[2] indicates that antimuscular dystrophy and antiparalytic factors exist, while Stockstad[3] reports evidence of a distinct growth factor U for the chick.

9 Amberson, W. R., *Biol. Rev.*, 1937, **12**, 48.

1 Morgulis, S., Wilder, V. M., and Eppstein, S. H., *J. Nutr.*, 1938, **16**, 219.

2 Jukes, T. H., and Babcock, S. H., *J. Biol. Chem.*, 1938, **125**, 169.

3 Stockstad, E. L. R., and Manning, P. D. V., *J. Biol. Chem.*, 1938, **125**, 687.

The work herein reported has been confined to the differentiation of the various factors of the B complex which are necessary for the rat. Three-weeks-old rats of 30 to 35 g weight were placed in groups of 6 in screen bottom metal cages and received weighed amounts of the supplemented diets furnishing the desired quantity of each supplement per day. The basal ration contained 60% alcoholic extracted washed casein 18.0, salt mixture 3.7, cod liver oil (Squibb) 4.0, and sucrose to 100%. Three and one-half percent of water was added to prevent rancidity. The washed casein was prepared by washing commercial casein 14 days with 0.15% acetic acid and decanting daily. From this casein was prepared the alcoholic extracted casein by extracting in a continuous extractor with 60% alcohol for 4 days.

Concentrates of filtrate I ($B_6$) and filtrate II (FII) were prepared from liver and from rice polish by the method of Lepkovsky, Jukes and Krause.[4] The $B_6$ concentrate was purified further by readsorption and elution before concentration. Both $B_6$ and FII fractions were found necessary in addition to $B_1$, flavin, and nicotinic acid for the growth and well being of the rat. Omission of nicotinic acid in diets with 0.3 g of alfalfa plus washed casein or with 0.3 g alfalfa plus 8B as a source of total filtrate resulted in decreased growth showing that nicotinic acid is essential for rat growth and that this substance is deficient in 0.3 g of alfalfa, in washed casein, and in 8B concentrate.

The following are the results of studies to investigate the possible multiple nature of FII. The adsorbate and filtrate obtained by treatment of the FII fraction with norite gave no supplementary effect to one another, although about ¼ of the total activity was adsorbed. Adsorbed and unadsorbed fractions prepared by continuous (chromatographic) adsorption of total filtrate from liver (filtrate from flavin adsorption) on 1 kg English fuller's earth or on 3 kg of this earth gave no significantly better growth when fed in combination as a source of FII than when fed alone. Less than ½ the activity was adsorbed on 1 kg of the fuller's earth and more than ½ on 3 kg of the fuller's earth.

When concentrates of $B_6$ and FII (prepared from liver) were fed together as a source of the total filtrate ($B_1$, flavin supplied) poorer growth and appearance of the animals resulted when crystalline $B_1$ was employed than when Feaster and Nelson's[5] fuller's earth 8B adsorbate from rice polish extract was used to supply $B_1$. Again, in

[4] Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

[5] Feaster, J. F., and Nelson, V. E., *Am. J. Physiol.*, 1936, **115**, 147.

groups receiving the adsorbed portion from the continuous adsorption treatment or the combination of adsorbed and unadsorbed portions as a source of FII a marked stimulation of growth resulted by the addition of the 8B concentrate.

The supplementary effect of the 8B concentrate was also strikingly shown when it was added to 0.3 g of alfalfa daily as the sole source of the total filtrate. Without 8B, 0.3 g alfalfa supplemented with adequate $B_1$, flavin, and nicotinic acid gave poor growth and the rats appeared unthrifty and exhibited alopecia and dermatitis. With 8B in place of crystalline $B_1$ growth was twice as rapid and the animals maintained a healthy appearance. The effect of 8B on the alfalfa diet was not due to its $B_6$ content as was shown by supplementing the alfalfa diet with a tested $B_6$ concentrate, in which case little or no beneficial effect resulted. The possibility that the 8B effect might be merely additive was eliminated when it was found that twice the level of 8B failed to support growth and well being as a source of FII, while twice the level of alfalfa did not give nearly as good results as the combination of alfalfa and 8B.

The use of washed casein in the alfalfa ration in place of alcohol extracted washed casein resulted in increased growth but hastened the development of dermatitis, indicating a growth factor and an antidermatitis factor may be involved in the supplementary effect of 8B.

Molasses (0.15 g) and yellow corn (0.375 g) were also found effective in supplementing 0.3 g alfalfa as a source of the filtrate factor ($B_1$, flavin, nicotinic acid added).

The results show that rats require at least 2 factors in addition to $B_1$, flavin, nicotinic acid, and $B_6$. One of these factors is supplied by the alfalfa; whereas the other factor is present in the fuller's earth adsorbate from rice polish extract (fraction 8B) as well as in molasses and yellow corn. The distribution of the 8B factor, the effects of its deficiency and its adsorbability indicate that it differs from the chick antidermatitis factor, the antiparalytic factor, and factors U and W.[6]

[6] Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **121**, 255.

10385

## Immunologic Studies on Patients Suffering from Bacterial Endocarditis.

MARY A. POSTON AND EDWARD S. ORGAIN. (Introduced by David T. Smith.)

*From the Departments of Bacteriology and Medicine, Duke University School of Medicine, Durham, N. C.*

That specific antibodies are present in the circulating blood of patients with *Streptococcus viridans* endocarditis has been accepted very generally[1, 2, 3] and Keefer[4] has recently reviewed the studies supporting this view. Unfortunately varying technics have been used by previous workers, and it is difficult to correlate their results. We present here a study of the antibody responses in bacterial endocarditis, using a uniform technic, and a correlation of these serological findings with the variations of the colony count of successive blood cultures.

The opsonocytophagic power of the blood,[5] agglutination,[5] and bacteriolysin tests* were done on 7 patients with classical, and one patient (Case 2) with suspected bacterial endocarditis. In addition complement fixation tests were performed on Case 7. Three to 7 positive blood cultures were obtained on each patient before the immunologic status was determined. The results before treatment are shown in Table I.

After the completion of preliminary tests for antibodies, the patients were treated by means of a high vitamin diet, with specific

---

1 Blumer, G., *Medicine*, 1923, **2**, 105.

2 Cecil, R. L., *Textbook of Medicine*, 4th edition, W. B. Saunders, Philadelphia, Pa., 1937, 1105.

3 White, P. D., *Heart Disease*, 2nd edition, The Macmillan Co., New York, N. Y., 1938, 263.

4 Keefer, C. S., *Ann. Int. Med.*, 1937, **11**, 714.

5 Menefee, E. E., Jr., and Poston, M. A., *Am. J. Med. Sc.*, in press.

* Felsen's[6] method for measuring bacteriolysins was modified as follows: After preliminary titration to establish the presence of complement, a series of test tubes containing 0.5 cc of different dilutions of the patient's serum (from 1-10 to 1-1000) was incubated in a 37°C water bath for 4 hours with an equal amount of suspension of organisms. Ten cc of beef infusion broth pH 7.4 (blood broth for *H. para-influenzæ*) was added to each tube and the amount of growth in 18 hours was determined by readings on a calibrated Photronreflectometer (Libby). One cc of the serum organism mixture was used for pouring plates with North's gelatin blood agar for *N. gonorrhoeæ*.

6 Felsen, J., and others, *J. A. M. A.*, 1937, **108**, 1783.

TABLE I.

| Case No. | Etiological agent | Before Treatment: No. of colonies per cc of blood* | Before Treatment: Opsonocytophagic power of the blood†‡ | Before Treatment: Agglutination† | Before Treatment: Bacteriolysins† | After Treatment: No. of colonies per cc of blood | After Treatment: Opsonocytophagic power of the blood‡ | After Treatment: Agglutination | After Treatment: Bacteriolysins | Duration before entry mos | Followed mos | Results |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *S. viridans* | 6 | 17 | Negative | None | Not done; treatment refused | | | | 3 | | Died |
| 2 | " " | 1 | 0 | " | " | 0 | 50 | 1-640 | 1-500 dil. of serum; no growth | 1.5 | 8 | Well |
| 3 | " " | 3 | 0 | " | " | 0 | 21 | 1-640 | 1-1000 dil. of serum; no growth | 6 | 6.5 | Died |
| 4 | " " | 23 | 5 | " | " | 1 | 1 | 1-640 | None | 2 | 2.5 | " |
| 5 | Enterococcus | 3 | 0.5 | " | " | 0 | 19 | 1-80 | " | 11 | 1 | Died of pulmonary embolus |
| 6 | *H. parainfluenzæ* | 1 | 0.4 | 1-80 | " | 0 | 0.5 | 1-80 | " | 4 | 1.5 | Died |
| 7 | *S. viridans* | 6 | 0 | 1-40 | " | 21 | 4 | 1-80 | " | 3 | 1 | Died 7 wks after discharge against advice |
| | *H. parainfluenzæ* (hemolytic) | 5 | 0.2 | 1-20 | " | 1 | 11 | 1-160 | 1-50 dil. of serum; no growth | | | |
| 8 | Anerobic streptococcus (non-hemolytic) | 1 | 0.5 | Negative | " | 0 | 93 | 1-1280 | 1-200 dil. of serum; no growth | 4 | 3 | Well |
| | *N. gonorrheæ* | 1 | 1 | 1-80 | " | 0 | 97 | 1-1280 | 1-200 dil. of serum; no growth | | | |

* Average of 3-7 blood cultures. † Average of 2-3 tests. ‡ Average number of organisms per cell.

vitamin supplements, iron, liver, multiple blood transfusions, and sulfanilamide, or one of its related compounds. Serial studies were done on each case over periods varying from 1 to 8 months. The maximal immunologic response after treatment is shown in Table I.

Initial studies in each instance revealed uniformly little evidence of antibodies either in the *Streptococcus viridans* or the other etiological types of endocarditis. In Case 2, the blood, after 3 consecutive positive cultures, became sterile, and immune antibodies previously absent appeared in high titer. In Case 3, immune antibodies appeared when the blood became sterile, but decreased sharply following injections of a formalinized autogenous vaccine. When the colony count in Case 4 decreased from 26 to 1 per cc, the agglutination titer rose to 1-640, but no increase in phagocytosis or bacteriolysins was noted. These agglutinins disappeared when the colony count increased to 45 per cc. In Case 5, after the disappearance of the bacteremia, phagocytosis and the agglutination titer rose before the patient's premature death from pulmonary embolism. Case 7 developed immune antibodies in low titer in the presence of a persistent bacteremia. Case 8 revealed a progressive rise in antibodies after the blood cultures became negative. Gonococcal complement fixation tests, initially 4 plus, later became negative, indicating possibly an inverse relationship to other antibodies. Kinsella[7] in one instance of *Streptococcus viridans* endocarditis, demonstrated an inverse relationship of agglutinins to complement fixing antibodies, but did not comment further upon this finding.

*Conclusions.* Patients in the active bacteremic stage of bacterial endocarditis exhibit no significant serologic evidence of immunity by the methods used in this study. Immune antibodies developed coincidentally with the disappearance of bacteremia.

---

[7] Kinsella, R. A., *Arch. Int. Med.*, 1917, **19**, 367.

10386

## Nitrate and Bromide Tests for Blood-CNS Barrier Permeability in Experimental Poliomyelitis.*

EDWIN H. LENNETTE, DAN H. CAMPBELL AND HAROLD R. REAMES.
(Introduced by G. M. Dack.)

*From the Department of Bacteriology and Parasitology, University of Chicago.*

Changes in the permeability of the blood-CNS barrier are known to occur in a number of pathologic conditions involving the central nervous system.[1] Of the many tests devised for measuring such changes in permeability, that of Mestrezat and Gaujoux,[2] based on the passage of nitrate ions through the barrier into the spinal fluid, is one of the simplest. This test, which is roughly quantitative, proved in our hands to be so crude that the results in any single instance were difficult to interpret; however, by employing a large series of monkeys we[3] have been able to demonstrate that an increased barrier permeability to sodium nitrate occurs in experimental poliomyelitis.

Because the crudeness and unreliability of the nitrate test far outweighed any advantages accruing from the simplicity of the analytical procedure, we[4] later replaced this method with the Walter bromide test,[5] adapted by us to meet the needs of experimental work on monkeys. This test was found to be much more accurate than the nitrate method; in fact, it was usually possible for the analyst, who had no previous knowledge of the clinical status of the animals tested, to report whether or not the animals were infected, something which could only occasionally be done with the nitrate test. For this reason we thought it desirable to compare the two tests, barrier permeability being tested on the same animal by both methods simultaneously.

The comparative tests were done on 54 *rhesus* monkeys *(Macaca mulatta)*, 33 of which were in the acute stages of poliomyelitis. The control series consisted of 10 normal, healthy monkeys and 11 animals which had been injected intracerebrally with 1.0 cc of normal

*This work was supported by a grant from the President's Birthday Ball Commission for Infantile Paralysis Research.

1 Katzenelbogen, S., *The Cerebrospinal Fluid and Its Relation to the Blood*, Baltimore, The Johns Hopkins Press, 1935.

2 Quoted by Loberg, K., *Z. f. d. ges. Neurol. u. Psychiat.*, 1926, **106**, 164.

3 Lennette, E. H., and Reames, H. R., *J. Immunol.*, 1938, **34**, 215.

4 Lennette, E. H., and Campbell, D. H., *Science*, 1937, **86**, 160.

5 Walter, F. K., *Z. f. d. ges. Neurol. u. Psychiat.*, 1925, **95**, 522.

monkey spinal fluid, 1.0% normal monkey cord suspension or 2.0% starch to determine the effect of trauma on the permeability of the barrier.

Sodium bromide was administered subcutaneously twice daily for 2 consecutive days, 45 mg per kg of body weight being given. On the 3rd day 35 mg per kg of sodium nitrate was injected intravenously. One hour later spinal fluid was withdrawn by cisternal tap, blood being obtained at the same time by cardiac puncture.† The nitrate content of a portion of the spinal fluid was determined by a method described elsewhere;[6] the remaining spinal fluid and the blood serum were analyzed for bromide by a modification[7] of the original Walter technic.

In Table I are summarized the results; the upper figures represent the mean of the determinations made, the lower figures the extremes of the range over which the determinations varied. The table shows that poliomyelitic monkeys tended to have a larger amount of nitrate in the spinal fluid than did the control animals; the extent of overlapping, however, was so great that unequivocal results were obtained only when barrier permeability was so marked that relatively large amounts of nitrate appeared in the spinal fluid. Essentially

TABLE I.
Comparison of the Nitrate and Bromide Tests for Measuring Blood-CNS Barrier Permeability in Experimental Poliomyelitis.

| History of monkeys | No. of monkeys | Conc.* $NaNO_3$ in spinal fluid | Conc.† of NaBr in | | Bromide P.Q. |
|---|---|---|---|---|---|
| | | | Spinal fluid | Serum | |
| Normals | 10 | 27.0 | 24.2 | 38.4 | 1.58 |
| | | 20-30 | 20.2-30.8 | 28.9-43.0 | 1.34-1.88 |
| Trauma controls | 11 | 29.1 | 30.0 | 36.6 | 1.22 |
| | | 20-40 | 22.2-33.2 | 29.3-41.7 | 1.15-1.33 |
| Preparalytic | 17 | 31.8 | 33.8 | 33.8 | 1.00 |
| | | 20-50 | 25.6-46.6 | 27.1-47.1 | .91-1.02 |
| Paralytic | 16 | 40.0 | 35.2 | 35.1 | .99 |
| | | 30-70 | 26.9-41.9 | 28.6-42.3 | .94-1.02 |

* Expressed in mg per liter.
† Expressed as mg %.

† Both procedures were done under deep ether anesthesia.

[6] Lennette, E. H., and Reames, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 769.

[7] Lennette, E. H., and Campbell, D. H., *Am. J. Dis. Child.*, 1938, **56**, 756.

the same was found to hold true with bromide when only the spinal fluid concentration was considered; when the degree of permeability, however, was expressed as a ratio (serum bromide/spinal fluid bromide), usually referred to as the permeability quotient or P.Q., the difference in barrier permeability between the various groups at once became apparent. It is probable that similar differences might be shown with the nitrate test were it more precise and were the method applicable to serum analyses.

It appears, then, that even exact determinations of the spinal fluid concentration of any substance used to test barrier function may give little information as to the degree of altered permeability present, except when permeability is so greatly increased that unduly large amounts of the test agent are permitted to pass the barrier. A more precise evaluation of barrier function can be achieved by determining the concentration of the test agent in both blood and cerebrospinal fluid, and expressing the degree of permeability as a quotient (P.Q.).

## 10387

## Change in Amino Acid Oxidation Following Dissociation of *Staphylococcus aureus.*

M. DOROTHY WEBSTER. (Introduced by C. C. Lieb.)

*From the Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina.*

Using the Methylene Blue technic, Bach[1] has reported that in the presence of *Staphylococcus aureus,* the amino acids with the exception of cysteine and glutamic acid are not active as hydrogen donators. The amino acids in the present study are classed by him as "doubtful or inactive." Soru[2, 3] compared the ability of the dissociated and undissociated *B. coli* to oxidize glucose. He found that the fermentation of glucose, various other substrates, and other sugars is slower with the R form than with the S form. Alanine and serine were so slowly oxidized that no difference in reduction time could be noted. Under similar conditions, using Bacille d'aertrycke, glycine was not attacked, and other substances such as

1 Bach, D., *Compt. rendu de Soc. Biol.,* 1935, **120**, 608.
2 Soru, E., *Compt. rendu de Soc. Biol.,* 1936, **121**, 1647.
3 Soru, E., *Compt. rendu de Soc. Biol.,* 1935, **120**, 232.

glucose, lactate, and succinate were oxidized more slowly by the R than by the S form.

*Staphylococcus aureus* was isolated from a case of clinical infection. The strain was hemolytic and the colony a bright yellow color. The bacteria were grown on beef extract agar at 37°C and pH 7.4. They were washed off the agar with saline and centrifuged. Washing was repeated 3 times, and the bacteria were then suspended in 0.05 M phosphate buffer, pH 7.8. Bacterial counts were done by means of a hemocytometer and it was found that there were approximately sixteen billion per cc.

Dissociation was brought about by aging the culture. Plates streaked on the 25th day showed many rough white colonies. This rough white dissociate, referred to hereafter as *Staphylococcus albus*, was subcultured and showed no tendency to revert to the smooth yellow form.

One cc of the suspension was used in each Warburg vessel and the volume made up to 2 cc with buffer and solution of the amino acid to be tested. The rate and the amount of the oxygen uptake were then measured. The amount of decarboxylation was determined by tipping in 0.5 M HCl at the end of the experiment. Deamination was measured by vacuum distillation followed by Nesslerization.

Of 13 amino acids tested, only 5, glycine, serine, alanine, proline, and histidine were attacked. Valine, leucine, isoleucine, hydroxyproline, tyrosine, phenylalanine, tryptophane, and methionine were not attacked.

The simpler amino acids, glycine, serine, and alanine were oxidized in the same manner as with *B. proteus*[4] and *B. pyocyaneus*.[5] Glycine was completely oxidized to carbon dioxide, ammonia and water. Both isomers of alanine were oxidized, taking up four atoms of oxygen per molecule of amino acid and producing ammonia and 2 molecules of carbon dioxide. Only the dl-mixture of serine was available, but the ammonia production indicated that both isomers were attacked. Three atoms of oxygen were utilized and one molecule of carbon dioxide was produced per molecule of serine. Dissociation had no apparent effect upon the ability of the bacteria to oxidize any of these simple amino acids.

With proline and histidine the extent of the oxidation was changed upon dissociation of the bacteria. *Staphylococcus aureus* oxidized only the natural isomer of proline, utilizing 8 atoms of oxygen per

[4] Bernheim, F., Bernheim, M. L. C., and Webster, M. D., *J. Biol. Chem.*, 1935, **110**, 165.

[5] Webster, M. D., and Bernheim, F., *J. Biol. Chem.*, 1936, **114**, 265.

TABLE I.

Oxidation, Decarboxylation, and Deamination of Various Amino Acids at 37°C and pH 7.8.

The oxygen uptake and decarboxylation as shown in Columns 4 and 8 are calculated for both isomers, when d-l forms were used. For determining the amount of deamination 1.0 mg was used throughout and the theoretical deamination as shown in Column 11 is calculated on that basis. The oxidation rate is based on the time necessary for half the final oxygen uptake to be reached.

| | | $O_2$ Uptake | | | | $CO_2$ Production | | | $NH_3$-N | |
|---|---|---|---|---|---|---|---|---|---|---|
| (1) Amino acid | (2) Amt. used mg | (3) Exper. $mm^3$ | (4) Theor. $mm^3$ | (5) Atoms | (6) Rate min. | (7) Exper. $mm^3$ | (8) Theor. $mm^3$ | (9) Mol. | (10) Exper. mg | (11) Theor. mg |
| Glycine | | | | | | | | | | |
| *S. aureus* | .25 | 94 | 111 | 3 | 27 | 132 | 140 | 2 | .172 | .186 |
| *S. albus* | .25 | 103 | 111 | 3 | 30 | 134 | 140 | 2 | .176 | .186 |
| dl-serine | | | | | | | | | | |
| *S. aureus* | .25 | 73 | 81 | 3 | 40 | 58 | 57 | 1 | .126 | .133 |
| *S. albus* | .25 | 76 | 81 | 3 | 15 | 46 | 57 | 1 | .123 | .133 |
| dl-alanine | | | | | | | | | | |
| *S. aureus* | .25 | 137 | 126 | 4 | | 133 | 128 | 2 | .147 | .158 |
| *S. albus* | .25 | 130 | 126 | 4 | | 116 | 128 | 2 | .150 | .158 |
| d-alanine | | | | | | | | | | |
| *S. aureus* | .25 | 129 | 126 | 4 | 10 | 134 | 128 | 2 | .145 | .158 |
| *S. albus* | .25 | 133 | 126 | 4 | 15 | 117 | 128 | 2 | .146 | .158 |
| dl-proline | | | | | | | | | | |
| *S. aureus* | .25 | 101 | 194 | 8 | | 77 | 196 | 4 | .055 | .121 |
| *S. albus* | .25 | 132 | 121 | 5 | | 106 | 98 | 2 | .098 | .121 |
| l-proline | | | | | | | | | | |
| *S. aureus* | .25 | 206 | 194 | 8 | 40 | 209 | 196 | 4 | .115 | .121 |
| *S. albus* | .25 | 121 | 121 | 5 | 48 | 109 | 98 | 2 | .098 | .121 |
| d-histidine | | | | | | | | | | |
| *S. aureus* | .25 | 18 | 0 | 0 | | 8 | 36 | 0 | .020 | .088 |
| *S. albus* | 1.00 | 4 | 0 | 0 | | 0 | 36 | 0 | .000 | .000 |
| l-histidine | | | | | | | | | | |
| *S. aureus* | .25 | 114 | 116 | 8 | 140 | 29 | 36 | 1 | .090 | .088 |
| *S. albus* | 1.00 | 62 | 58 | 1 | 120 | 73* | 72* | 1 | .077 | .088 |

*.5 mg used.

molecule and producing 4 molecules of carbon dioxide. *Staphylococcus albus* attacked both isomers of proline but only 5 atoms of oxygen were utilized and 2 molecules of carbon dioxide produced per molecule of amino acid. The ammonia production indicated that only one isomer was attacked by the original strain and both isomers by the dissociate form.

Histidine in the presence of *Staphylococcus aureus* is oxidized, taking up 8 atoms of oxygen and producing one molecule of free carbon dioxide. Only the natural isomer is attacked. With *Staphylococcus albus* only one atom of oxygen is utilized per molecule of amino acid and one molecule of carbon dioxide produced. In this case also only the natural isomer is attacked.

All the reactions tested were sensitive to 0.005 M KCN.

The course and extent of the oxidation of the simpler amino acids, glycine, serine, and alanine by *Staphylococcus aureus* and *Staphylococcus albus* is the same as with *B. proteus* and *B. pyocyaneus*. It would, therefore, seem likely that there is a common mechanism for the carrying out of these oxidations. A change in the course and extent of the oxidation of proline and histidine takes place when *Staphylococcus aureus* is dissociated. Whether this change is significant in the problem of virulence and toxin production has not yet been determined. The ability to oxidize a given amino acid varies from species to species Thus, the oxidation of phenylalanine by *B. proteus*[4] utilizes one atom of oxygen, by *B. pyocyaneus*[5] 13 atoms while there is no oxidation by *Staphylococcus aureus* or *Staphylococcus albus*.

*Summary.* 1. The amino acid oxidation by *Staphylococcus aureus* and the rough white dissociate *Staphylococcus albus* has been studied. 2. Glycine, serine, and alanine are oxidized in the same fashion by both forms. 3. *Staphylococcus aureus* exhibits optical specificity in its oxidation of proline, in that only the natural isomer is attacked. After dissociation, the optical specificity is lost and both isomers are equally oxidized, but the extent is not as great as by the undissociated form. The optical specificity with regard to histidine is maintained after dissociation, but the extent of the oxidation is much reduced.

I wish to express my thanks to Doctor D. S. Martin of the Department of Bacteriology and to Doctor Frederick Bernheim of the Department of Physiology and Pharmacology of Duke University Medical School for their constant help and advice throughout the course of this work.

10388

## Filtrability of the Chromogenic Culture (Duval) Obtained from Human Leprosy.

WALKER L. LOVING AND JOSEPH R. KRIZ. (Introduced by C. W. Duval.)

*From the Department of Pathology and Bacteriology, School of Medicine, Tulane University, New Orleans, La.*

Within recent years much has been written regarding filtrable forms of bacteria, especially by Hadley, Blanc, Wolbach, Tunnicliff, and Duval. The literature reveals but few reports upon work dealing with the filtrability of acid-fast cultures recovered from the human leprous lesion. It occurred to one of us (Kriz) that *B. lepræ* chrome (Duval)[1] might under certain environmental conditions exhibit a filtrable stage. Walker,[2] and Sweeney[3] state that they obtained a filtrable form of the bacilli of human and rat leprosy, using filtrates from tissue-emulsions. These authors claim to have cultured from the filtrates a facultative acid-fast microörganism comparable in morphology to the bacilli in the respective tissue-lesions. The bacilli growing out from planted filtrates exhibited a wide range of morphology. Non acid-fast coccoid forms later became acid-fast bacillary types, which according to Walker and Sweeney indicate not only pleomorphism but bio-chemical inconstancies of the microörganisms with which they worked. This feature is of course not new for any acid-fast microbe. Kedrowski[4] has long maintained that there are wide variations in morphology and tinctorial properties with respect to acid-fastness for his culture from the human leprous lesion. Markianos[5] claims to have obtained a filtrable form of *B. lepræ muris* from rat tissues, with which he claims to have produced experimental leprosy in these animals.

We have tested culture-filtrates of both old and recently isolated strains of the chromogenic acid-fast bacillus (Duval). After obtaining good growth on glycerine agar, a portion of the surface growth was transferred to separate flasks containing 400 cc of glycerine broth at pH 7.4. After 2 weeks' incubation there was a

1 Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.

2 Walker, E. L., and Sweeney, M. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1162.

3 Walker, E. L., and Sweeney, M. A., *J. Inf. Dis.*, 1935, **56**, 97.

4 Kedrowski, W., *Z. f. Hyg. u. Infekionskr.*, 1901, **27**, 52.

5 Markianos, P. J., *Bull. Soc. Path. Exot.*, 1929, **22**, 410.

marked increase in turbidity. At weekly intervals, over a period of 11 weeks, 20 cc from each flask were passed through a Seitz filter under a pressure of about 20 mm of mercury. Just immediately preceding filtration, preparations were made and stained to determine morphological features and acid-fastness of the bacilli. Two cc portions of the filtrates were transferred to glycerine-agar slants in tubes plugged with paraffined cotton and topped with tinfoil to prevent drying of the medium, and incubated at 37.5°C. At no time was there macroscopical or microscopical evidence of growth over the entire period of observation (3 months).

*Summary.* It would appear from the work of others that a filtrable form of *B. lepræ* exists *in vivo* for the respective bacilli of human and rat leprosy, since *in vitro* growth is said to have occurred from the cultured filtrates. Employing filtrates of cultures of *B. lepræ chrome* (Duval) we have been unable after repeated attempts to demonstrate a filtrable stage of this microörganism.

10389

## Variations in Pulmonary Vital Capacity in Health: Daily, Seasonal, and at Moderate Altitudes.

FRANK L. APPERLY. (Introduced by H. B. Haag.)

*From the Department of Pathology, Medical College of Virginia, Richmond.*

*A. Daily and Seasonal Variations.* Occasionally writers draw various deductions from small alterations in the vital capacity of their patients. Anyone, however, with any experience in spirometric work is aware of the daily variations in healthy people, which may amount to several hundred ccm. These are usually disregarded or explained as due to temperature and pressure. I am not aware, however, of any observations of such variations over prolonged periods.

When air is taken into the lungs it is not only heated to a temperature close to that of the body but it becomes charged with water vapor. When this air is expelled with all the forces of expiration into a spirometer at ordinary room temperature and pressure we would expect: (a) A contraction of volume with perhaps condensation of water vapor resulting in a spirometric reading less than the true vital capacity, an error which would increase with falling temperatures, and (b) the final volume of the mass of air forcibly ex-

pelled into the spirometer to vary inversely as the atmospheric pressure opposing its expulsion.

Observations of vital capacity (largest of 3 trials), laboratory temperature and barometric pressure were made several times each month for a year in 2 individuals, and for a part of the year in 6 others. Fig. 1, showing the results over a period of 7 months, is typical of all. (These observations were made in Melbourne, Australia, hence the inversion of the temperature record between February and August.)

*Results.* (1) The daily and the general directions of the vital capacity curve follow that of the temperature curve, swinging down in winter to rise again the following summer. This is not in agreement with the statement of Griffith, *et al.*,[5] that "neither the vital

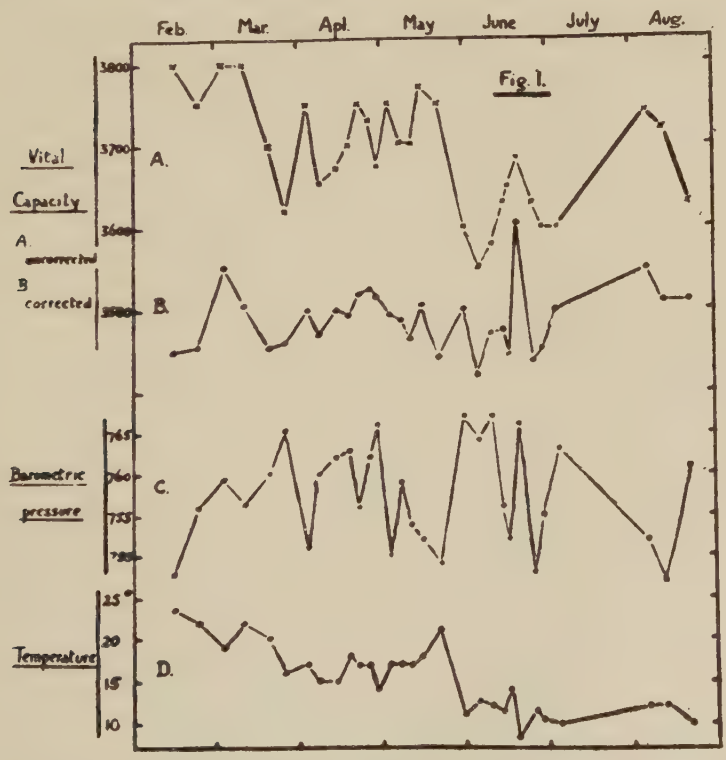



FIG. 1.

Variations in pulmonary vital capacity, barometric pressure and temperature over a period of 7 months in one subject. Curve B represents the results after correction of curve A for temperature and barometric pressure.

5 Griffith, F. R., Pucher, G. W., Brownell, K. A., Kline, J. D., and Carmer, M. E., *Am. J. Physiol.*, 1929, **89**, 555.

capacity nor the respiratory rate . . . . has a seasonal variation." (2) The daily vital capacity volumes vary inversely as the barometric pressure, these two curves being almost the mirror image of each other. (3) When we correct for variations due to temperature and pressure, reducing all volumes to standard conditions, (0°C and 760 mm Hg) we find the volume curve levels off at about 3500±50 ccm. It is probable that these variations are due entirely to experimental error, since the shape of the curve is now entirely independent of temperature or pressure, *i. e.*, there cannot be any real changes in lung capacity due to either of these factors. This is in contrast to our results in section B, below.

*B. Variation at Moderate Altitudes.* A number of observations were also made on a group of 4 individuals before, during and following a one-day visit to an altitude of about 3000 feet, and on another group of 6 people during a 9-day sojourn at 6000 feet. The journeys were made by automobile, partly to avoid possible errors due to exercise and fatigue. The same spirometer was used on all occasions. Since the 2 groups were partly composed of different

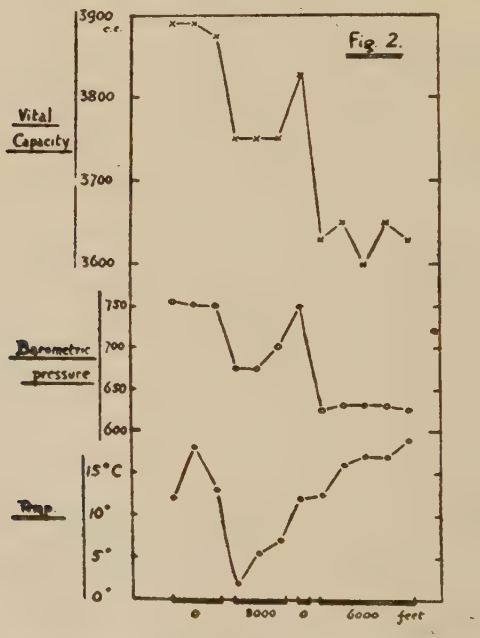



FIG. 2.

Variations in pulmonary vital capacity, barometric pressure and temperature at sea-level, 3000 feet and 6000 feet respectively, in one subject. (Note that the divisions of the barometric scale correspond to 50 mm Hg, *i. e.*, are 10 times those in Fig. 1.)

people it is not possible to compare their average figures. Fig. 2 shows the results in one individual who accompanied both groups, and his results are typical of all.

*Results.* Although, as shown in section A above, vital capacity volumes varied inversely as the small variations in barometric pressure at sea-level, we now find that vital capacity varies directly with the much larger changes in barometric pressure due to moderate altitudes. (Note that the barometric pressures are plotted in Fig. 2 on a scale 10 times greater than those in Fig. 1.) Furthermore, at 6000 feet there was a rising temperature, a condition which at sea-level we found to increase spirometric readings. At this level the vital capacity falls to 3600-3650 cc which, at standad conditions is reduced still further to 2800-2900 cc, a large reduction in oxygen supply.

The diminution of vital capacity at high altitudes has been noted by many others.[1, 6, 8] Zuntz[10] believed it to be due to expansion of intestinal gases, but Schneider[8] showed that there is no increase of abdominal girth. Durig[3] blamed fatigue of respiratory muscles and Fuchs[4] regarded it as the result of increased muscular tone due to cold. The last 2 possibilities were certainly absent in our experiments. Mosso's view,[7] that there is a dilatation of lung capillaries with some congestion and consequent diminution of air space, seems best to fit the facts. Thus a diminution of vital capacity can easily be brought about by lying down, or bandaging the limbs, and conversely vital capacity can be increased by subjecting men to increased atmospheric pressure (Schilling[9]) and by venesection (Budelmann[2]). Schneider,[8] experimenting with subjects in a low pressure chamber, believes that no fall in vital capacity occurs until the pressures fall to the equivalent of 10,000 to 15,000 feet, and further, that part of the effect is due to an anoxic loss of vascular tonus. Our observations, however, show a decreased vital capacity at 3000 and

1 Bert, P., *La pression barométrique*, Paris, 1878.

2 Budelmann, G., *Klin. Wochschr.*, 1937, **16**, 704, 1711.

3 Durig, A., *Physiologische Ergebnisse der im Jahr 1906 durchgeführten Monte Rosa Expedition*, Vienna, 1909.

4 Fuchs, R. F., *Sitzungsb. Physick. Med. Soz., Erlangen*, 1908, **40**, 238.

6 Grollmann, A., *Am. J. Physiol.*, 1930, **93**, 19.

7 Mosso, A., *Life of Man on the High Alps*, London, 1889.

8 Schneider, E. C., *Am. J. Physiol.*, 1932, **100**, 426.

9 Shilling, C. W., Hansen, R. A., and Hawkins, J. A., *Am. J. Physiol.*, 1935, **110**, 616.

10 Zuntz, N., Loewy, F., Müller, F., and Caspari, W., *Höhenklima und Bergwanderungen*, Berlin, 1906.

6000 feet in all subjects, at which altitudes the question of anoxia can hardly arise. The simplest explanation is that the fall of atmospheric pressure, by removing some of the external support of the pulmonary capillaries, allows dilatation thereof, resulting in a larger volume of blood and a smaller volume of air in the lung. Such capillary dilatation, although increasing the surface of blood exposed to alveolar air, diminishes the surface: mass ratio for pulmonary blood as well as the vital capacity. Blood examinations, however, show that both of these are well compensated by increased ventilation.

It is possible that the increased mass of blood in the lungs may be one of the factors favorably influencing certain forms of pulmonary tuberculosis at moderate altitudes.

*Summary and Conclusions.* 1. Apparent variations in pulmonary vital capacity are found from day to day and from season to season. When corrections are made for temperature and atmospheric pressure, these variations disappear. 2. True vital capacity diminishes at moderate altitudes, changes appearing even at 3000 feet. The various factors to which these changes have been attributed by different observers were absent in our experiments. Diminished vital capacity at altitudes is believed to be due to dilatation of lung capillaries following the partial loss of external (atmospheric) support, with consequent diminution of air space. Such an increase of blood in the lungs may be a factor in the favorable effect of moderate altitudes on certain forms of pulmonary tuberculosis.

**10390**

## Variability of Tubercle Bacillus. I. Mucoid Phase of the Human-Type Strain H-37.

RUTH A. MCKINNEY AND RALPH R. MELLON.

*From the Western Pennsylvania Hospital, Institute of Pathology, Pittsburgh, Pa.*

The characteristic attributes of both the rough and the smooth phases of the human tubercle bacillus have been amply recorded, but there has been no indication of the existence of a mucoid phase such as occurs in the dissociative pattern of many other microörganisms.[1] The isolation and subsequent growth of a mucoid phase in the human tubercle strain, H-37, is therefore briefly presented.

[1] Hadley, P. B., *J. Inf. Dis.*, 1937, **60**, 129.

The parent strain was a stock culture of H-37 (intermediate rough phase) on Petragnini egg-medium kept under paraffin seal at room temperature 4 months. The growth was dry, rough, crumbly; the organisms were pleomorphic rods, some being hooked or curved, others showing Y-formation or lateral budding. Many rods contained large granules and all organisms were strongly acid-fast.

The first experimental transfers from this growth were made to 3 liquid mediums: (1) a synthetic medium, pH 7.2, (2) 5% glycerol broth, pH 7.6; (3) a double-strength veal-infusion broth, resterilized, with 2% neopeptone (Difco), 5% glycerol, 0.04% asparagin-l, pH 8.0. The transfers were made, not to the surface, but to the bottom of each flask of liquid. In the first 2 mediums the growth was very slow and finely granular, with no later turbidity or pellicle-formation. The organisms were coherent or discrete beaded acid-fast rods. Repeated transfers from these mediums to a variety of solid mediums produced the dry, rough growth only, typical of culture H-37 containing short acid-fast rods.

The growth in neo-peptone glycerol-broth, pH 8.0, at first resembled that in the other 2 mediums. On the 9th day of incubation at 37°C, however, there developed a slight cloud above the granular growth, and on the 10th day the fluid was turbid. A needle withdrawn from the fluid carried with it material that formed a filament 2 feet long before snapping from the surface of the broth. The organisms in the granular growth were short acid-fast rods with very large terminal granules; those in the supernatant fluid were non-acid-fast, except for a slight retention of the stain (carbol-fuchsin decolorized with 5% nitric-acid-alcohol) in the granules only. Counterstained with Loeffler's methylene blue, the organisms were granular diphtheroid rods, some having clubbed ends. These organisms transferred to Loeffler's serum and to blood agar produced a confluent, almost clear mucoid growth with the consistency of egg-albumen. Most of the individual organisms were composed of large, centrally placed, deeply staining granules in short rods massed together in a matrix of amorphous material. In repeated transfers on Loeffler's serum the growth remained mucoid; the organisms became longer, polar-stained granular, non-acid-fast rods.

The mucoid growth on 2 other solid mediums merits description. One is an agar medium prepared from the filtrate of the growth, in alkaline neo-peptone glycerol-broth, of a "Critical Rough" strain of tubercle bacillus. The peculiar properties of the tuberculin produced by this organism have been described.[2] The pH is determined

[2] Mellon, R. R., Beinhauer, L. G., *Arch. Derm. and Syph.*, 1937, **36**, 515.

(7.2-8.0), 2% agar, 0.04% asparagin-*l*, at times 1% dextrose, are added. This medium is called CR agar. The other medium is a combination of the formulas of Löwenstein[3] and of Herrold,[4] omitting the glycerol in the former and substituting neo-peptone for bacto-peptone in the Herrold formula. This medium also contains dextrose and is referred to as LH agar. These mediums in this and other work have been very useful in enforcing and maintaining distinct coccal phases of the tubercle bacillus, which will be described in detail in another communication.

On the CR agar—and especially with dextrose—the mucoid growth is abundant, occurring typically in a peripheral wall-like formation about the colony. On standard glycerol agar the growth is more scanty, and rubbery in consistency. On the very alkaline CR agar the mucoid phase, after suitable streaking, has given rise to a scantily growing, very minute dry colony which pits the medium. The organisms are not miscible in water and stain poorly. They are non-acid-fast, short diphtheroid rods. The growth of the second diphtheroid in the dry colony is very sparse on the alkaline CR agar, less so on more acid CR agar, absent on standard glycerol agar.

On the LH agar a slow, but definitely spreading growth of the mucoid phase occurs. The center of the growth eventually is viscid and firm—the edge is more fluidly mucoid. The organisms in the two portions differed in the first transfer to this medium. The centrally located organisms were granular, non-acid-fast diphtheroid rods; those in the advancing peripheral growth exhibited remarkable pleomorphism—large, heavily granular organisms, with budding, branching, and terminal clubs—in short, a distinctly fungoid appearance. Moreover, nearly all of the organisms showed a partial return to the acid-fast condition, single structures retaining both acid-fast and non-acid-fast stain, especially noticeable in and near the club-formations which appeared to be the actively growing portion of the organism. On later transfers to the same medium the bacilli have become progressively more acid-fast and approach the pleomorphic picture of the original inoculum.

Repeating the original experimental transfers with freshly growing strains of H-37 on Petragnini medium has resulted in nothing but the usual rough growth in any medium used, either liquid or solid. But an examination of old stock strains of H-37 stored on Petroff's gentian-violet egg-medium in the cold for 6 to 9 months revealed a softening, gummy growth with a markedly pleomorphic

[3] Löwenstein, E., *Deutsche med. Wchnschr.*, 1930, **56**, 1010.

[4] Herrold, R. D., *J. Inf. Dis.*, 1931, **48**, 236.

morphology. *Such cultures only* have produced the mucoid growth in the alkaline neo-peptone glycerol broth, a growth which, so far, has not occurred in any other liquid medium tested. Further work is in progress as to the exact rôle played by this medium and the factors responsible for bringing to maturity the dissociation of the mucoid phase, which appears to be initiated in the aging cultures on Petroff's or other egg-mediums. Steenkin[5] has also noted the relationship between softening growth and pleomorphic rods of old tubercle cultures on egg-mediums; and particularly the eventual outgrowth of variants from the softened areas which, in his experience, however, were not mucoid.

10391 P

## Magnesium and Calcium in Striated Muscle as Revealed by the Electron Microscope.*

Gordon H. Scott and Donald M. Packer.

*From the Department of Anatomy, Washington University, St. Louis.*

Several years ago McMillen and Scott[1] described a simple magnetic electron microscope designed for use in localizing certain inorganic salts in biological tissues. After some modifications of this apparatus, mostly in the direction of increasing the magnification,[2] and much experimentation it has been possible for us to localize magnesium and calcium in tissues. Striated muscle is an appropriate tissue for the purpose since it has been shown by other methods that the salt distribution is orderly and precise.[3, 4]

The nearest approach to chemical conditions obtaining in the living is had by examination of tissues prepared for study by the Altmann-Gersh frozen dehydration method. Sections of tissues fixed by a modification of this technic were attached to a barium and strontium coated cathode and placed in the electron microscope. After evacuation of the instrument the cathode was heated slowly and the section vacuum-distilled until only the inorganic salts remained. The

5 Steenkin, W., Jr., *Am. Rev. Tuberc.*, 1938, **38**, 777.

* Aided by grants from the Rockefeller Foundation and the Josiah Macy, Jr., Foundation.

1 McMillen, J. H., and Scott, Gordon H., *Rev. Scientific Inst.*, 1937, **8**, 288.

2 Scott, Gordon H., and Packer, D. M., *Anat. Rec.*, 1939, in press.

3 Scott, Gordon H., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 349.

4 Scott, Gordon H., *Am. J. Anat.*, 1933, **53**, 243.

temperature of the cathode was raised further until active electron emission occurred. The electron source was imaged upon the fluorescent screen with appropriate magnetic lenses. Photographs were then made of the luminescent image.

After many tests with pure salts of magnesium and calcium it was determined that the electron source, responsible for the image, was these elements. Examination of such preparations enabled us to localize with some accuracy magnesium and calcium in striated muscle from several mammalian species. Due to the preliminary freezing process muscle is thrown into many strong contractions. In every case we could localize magnesium and calcium in these contraction bands. Little or none of these elements was present in the remainder of the sarcoplasm. It is also of some interest that there was no magnesium or calcium detected in the "tissue spaces" of Fenn.[5] Apparently the bulk of these elements existing in muscle is to be found in the muscle cells and not in the connective tissue. Naturally some must be present there since both magnesium and calcium must be transported to and from capillary and muscle cell.

## 10392 P

### Preliminary *in vitro* Studies of Melanophore-Principle Activity of the Pituitary Gland.*

GERALD FOSTVEDT. (Introduced by E. M. K. Geiling.)

*From the Department of Pharmacology, University of Chicago.*

An attempt was made to correlate the melanophore-dispersing hormone of the hypophysis with melanin production through the use of a known oxidase system (tyrosine-tyrosinase). The oxygen consumed in this system was measured with the Warburg apparatus. The melanophore-containing fractions were prepared by a modification of Stehle's method.[1] Acetone-desiccated powders prepared from (a) anterior and (b) posterior lobe beef pituitary glands, and (c) from the anterior lobe of sperm whale pituitary glands served as source material for the preparation of the various melanophore

[5] Fenn, W. O., *Symposia on Quantitative Biology*, Biological Laboratory, Cold Spring Harbor, N. Y., 1936, **4**, 252.

* Aided by a grant from the Rockefeller Foundation and from the Biological Research Funds of the University of Chicago.

[1] Stehle, R. S., *J. Pharm. Exp. Therap.*, 1936, **57**, 1.

fractions used in this study. Melanophore-dispersing activity of each fraction was assayed by injecting 1 cc doses of varying dilution into hypophysectomized frogs, *Rana pipiens*.[2] Hormone fractions obtained from whale anterior pituitary had activity in dilutions as high as $1 \times 10^{-6}$. Pressor and oxytocic hormone assays were run on the melanophore fractions obtained from posterior beef pituitary powder because of the known inhibitory effects of these hormones on melanophore activity.

For the enzyme, a tyrosinase solution was prepared by triturating 2 g of meal worms in 10 cc of buffer solution and centrifuging out extraneous material. Such solutions were of a crude nature and made up a part of the substrate, hence more gas was consumed than would theoretically be taken up in these oxidations. This was checked by adequate controls. The substrate used was recrystallized tyrosine. It was dissolved in a phosphate buffer at a pH of 6.85, or 7.33. Most of the experiments were conducted at a pH of 6.85 since the enzyme activity is slower in this range. All solutions of hormone, enzyme, and substrate were freshly prepared before each determination. The temperature of the bath was 38°C. The typical experiment consisted of 1.5 cc of .0033 molar tyrosine, 0.3 cc of tyrosinase solution, and 0.3 cc of a $1 \times 10^{-3}$ solution of melanophore hormone, the controls containing 0.3 cc of distilled water in place of the hormone solution.

The presence of the melanophore hormone from posterior beef pituitary in the reaction mixture was associated with a 12% increase in gas uptake over the control determinations by the end of a 5½ hour period. Destruction of the pressor and oxytocic hormones by alkali previous to use in this system, increased the gas uptake to 54% more than that of the controls by the end of the same 5½ hour period. This indicated that the pressor and oxytocic hormones were antagonistic to the accelerating effect which the melanophore hormone had on the oxidase system. Melanophore hormone obtained from freshly removed anterior lobes of beef pituitary showed a 30% increase in gas uptake by the end of 6 hours. Injection of the melanophore hormone fractions from anterior and posterior lobes of beef pituitary into hypophysectomized frogs showed a marked difference in reactivity. Apparently the presence of the melanophore hormone, over a wide dilution range, will accentuate the oxidase system. This latter point is being further investigated. A

2 Teague, R., Noojin, R., and Geiling, E. M. K., *J. Pharm. Exp. Therap.*, 1939, **65**, 115.

melanophore fraction of the anterior lobe of sperm whale[3] has been found to accelerate this oxidase system more than 100% in a period of 4 hours. Addition of standard pituitary solution decreased this acceleration 36%, due to the presence of the pressor and oxytocic principles. Further information as to the inhibitory nature of the pressor and oxytocic hormones was obtained when 0.3 cc of an acetic acid extract of the neural lobe of the sperm whale, which contains pressor and oxytocic hormones, but no melanophore hormone, decreased the oxidation rate of this oxidase system 12% as compared to the controls at the end of a 3-hour period. Destruction of the melanophore-containing fraction by tryptic digestion previous to use in the oxidase system resulted in a failure to accelerate this system and also indicated that the melanophore hormone did not act as additional substrate. The melanophore hormone alone has not been found to act as a substrate for tyrosinase. However, in a tyrosine-tyrosinase-melanophore hormone system the melanophore hormone is invariably partially destroyed during the oxidation of the substrate, as determined by injecting the resulting melanin product into hypophysectomized frogs. The addition of 8-hydroxy quinoline to the system prevents the oxidation even when the melanophore hormone is present.

The results of these experiments are being correlated with investigations on the thermostable metabolic stimulant that has recently been cited by O'Donovan and Collip[4] as being present in pituitary preparations rich in melanophore principle. The experiments so far completed point to at least one possible physiological rôle which the melanophore hormone may play in higher animals, namely, a stimulation of an oxidase system or systems in the body. It also indicates a probable rôle of this hormone in lower forms. The influence of this hormone on other oxidase systems is now being studied.

*Summary.* (1) *In vitro* experiments indicate that one of the biological mechanisms of the melanophore hormone is that of an accelerator of an oxidase system such as tyrosine-tyrosinase. (2) The pressor hormone, and perhaps the oxytocic hormone also, act to inhibit this oxidase system. (3) Melanophore hormone has been obtained from anterior sperm whale pituitary which results in a black coloration of hypophysectomized frogs when 1 cc of a $1 \times 10^{-6}$ dilution of this hormone is injected. The black coloration persists for at least 24 hours.

[3] Geiling, E. M. K., *Bull. Johns Hopkins Hosp.*, 1935, **62**, 123.

[4] O'Donovan, D. K., and Collip, J. B., *Am. J. Physiol.*, 1938, **123**, 157 (Proc.).

10393

## Acid-Base Balance and the Distribution of Fat in the Blood.

R. Levine and Samuel Soskin.

*From the Department of Metabolism and Endocrinology,* Michael Reese Hospital, and the Department of Physiology, University of Chicago.*

The mechanism of fat transport from and to the tissues is obscure. The fat content of the blood has been determined both experimentally and clinically in a variety of conditions, by a number of investigators.[1] It has been generally assumed that an increased amount of fat in the blood signified the transfer of fat from one site to another for the purposes of storage or metabolism. A review of this subject suggested to us that most of the conditions in which lipemia had been reported were characterized by the tendency for the acid-base balance to be shifted towards the acid side. This parallelism has been previously noted in the case of diabetes.[2, 3, 4] However, the same relationship is evident if one considers such clinical states as starvation, hemorrhage and nephritis and such experimental conditions as severe exercise, pancreatic diabetes and phlorhizination.[1] It, therefore, seemed worthwhile to investigate the effects of experimentally induced changes in acid-base balance upon fat transport as indicated by lipemia.

"Acid" and "alkaline" salts were administered intravenously or by mouth to normal dogs. Various salts were used (ammonium chloride, ammonium lactate, sodium bicarbonate and sodium lactate) to obviate the influence of specific ions. Blood samples were taken at hourly intervals for 4-6 hours after the salt administration and analyzed for total blood fat[5] of the whole blood and serum respectively, and for carbon dioxide-combining power. Hematocrit readings were made to determine the partition of the fat between the red blood cells and the blood plasma.

During the course of these experiments it became of interest to determine whether particular organs were important to the results which we obtained on normal animals. Accordingly the above pro-

* Aided by the Max Pam Fund for Metabolic Research.

1 Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry (Interpretations)*, Springfield, 1932.

2 Gunnar Blix, *Studies on Diabetic Lipemia*, Lund, 1925.

3 Allen, F. M., *J. Metab. Research*, 1922, **2**, 238.

4 Curtis, A. C., Sheldon, J. M., and Eckstein, H. C., *Am. J. Med. Sciences*, 1933, **186**, 548.

5 Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

cedures were performed on depancreatized, hypophysectomized, and eviscerated animals respectively. In the last mentioned group of animals an anesthetic was necessary. However, control experiments on anesthetized normal animals showed that the anesthetic (nembutal) employed did not influence the results.

The results of changes in the acid-base balance, by whatever means, were uniformly consistent in all our experiments. It will, therefore, suffice to present 2 typical experiments (Fig. 1) and a composite graph summarizing our results as a whole (Fig. 2). Since the control values for blood fat varied between wide limits in different animals, our results in Fig. 2 are expressed as percentage rise or fall from the control values.

The data show that changes in the acid-base balance have no significant influence on the fat content of the whole blood. It is evident, however, that a fall in the carbon dioxide-combining power is associated with a rise in the serum fat, while a rise in the carbon dioxide-combining power is accompanied by a fall in the serum fat. These results must be interpreted as indicating a shift of fat from the red blood cells to the plasma and vice versa, under the influence of disturbances in the acid-base equilibrium. The presence or absence of the pancreas, the hypophysis or the liver and abdominal viscera had no influence on the nature of the results obtained.

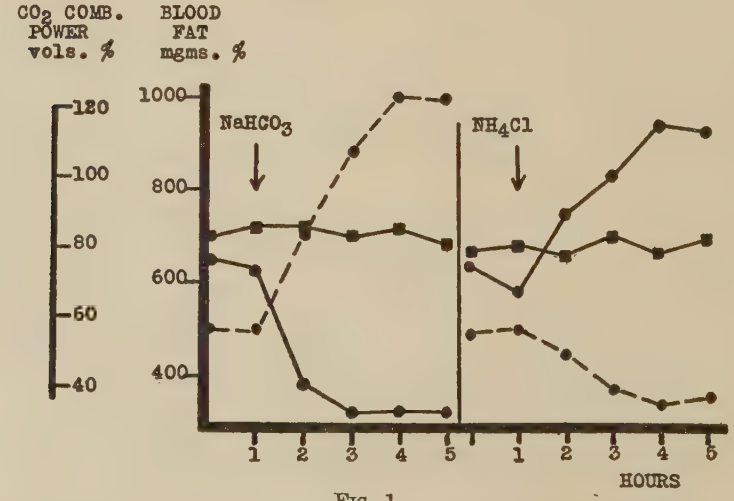



Fig. 1.

Two experiments on the same normal dog, showing the influence of experimentally induced increase and decrease of the carbon dioxide combining power upon the whole blood fat and serum fat respectively. The broken lines indicate carbon dioxide combining power. The continuous lines with the solid squares represent the whole blood fat. The continuous lines with the round dots represent the serum fat.

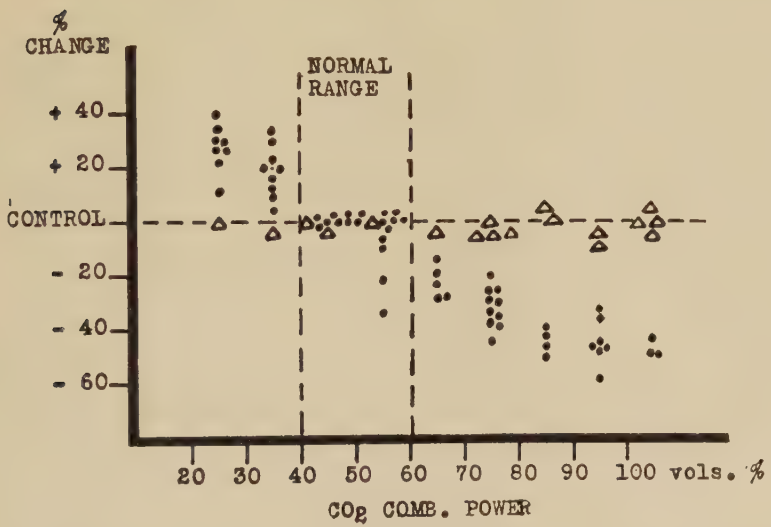



FIG. 2.

Summary of all our results in percentage change from the control values, to obviate the large normal variation in the latter. The round dots represent serum fat. The hollow triangles represent whole blood fat. Note that the serum fat is influenced by the carbon dioxide combining power, while the whole blood fat is not.

The term "lipemia" has been used indiscriminately to denote increases in the fat content of the whole blood, the plasma or the serum according to which medium the observers happened to direct their attention. It is evident from our results that increases in fat content of the whole blood and serum are not necessarily parallel, nor do they have the same significance as regards fat transport. A lipemia of the whole blood must indicate a transfer of fat from the tissues to the blood, and if the increased blood fat is carried to a distant organ or tissue, this would constitute the transport of fat. It is equally clear, however, that when fat is determined in the serum only, any lipemia so observed may indicate only a shift of fat across the membrane of the red blood cell into the plasma, without any increase in the total fat of the blood, and without any participation of the fixed tissues.

A review of the literature on lipemia, in the light of our present results, does not allow an unequivocal answer to the question of the relationship between the acid-base balance and fat transport. This is due to the fact that there has been no uniformity in the methods of fat analysis or in the use of whole blood or serum for the determinations.[6] Our results indicate that those determinations which

[6] Nissen, N. I., *Alimentary Lipæmia in Man*, Copenhagen, 1933. (Contains extensive bibliography on lipemias.)

were made on serum cannot be used to establish the relationship and, for the short experimental periods which we used, no transport of fat was observed. It is apparent that further work, using analysis of whole blood, is necessary. It may be noted, however, that even the shift of fat from red blood cells to plasma may have some significance as regards fat transport, since it represents a higher concentration of fat in that portion of the blood which is immediately concerned with the transfer of food stuffs to the tissue cells.

*Conclusions.* 1. A reciprocal relationship has been shown to exist between the carbon dioxide combining power of the blood and the concentration of total fat in the blood serum. 2. Changes in serum fat content produced by altering the acid-base balance are not accompanied by changes in the fat content of the whole blood, within the duration of our acute experiments. 3. The changes in serum fat are therefore the result of a shift of fat between the red blood cells and the blood plasma. 4. The significance of serum and whole blood lipemias as regards fat transport, is discussed.

10394

## Effect of Certain Yohimbine Derivatives upon Arterial Strips.

Harold F. Chase, Fredrick F. Yonkman and Albert G. Young. (Introduced by F. H. Pratt.)

*From the Department of Pharmacology, Boston University School of Medicine, and the Evans Memorial Hospital, Boston, Mass.*

Previous work by F. Meyer,[1] Hamet,[2, 3] Weger[4] and others indicates that the yohimbine radicle exhibits a rather marked antisympathicomimetic action in various organs. Some investigators have studied various isomers of yohimbine with similar results but our present work deals with several newly formed HCl salts of the yohimbine radicle in the form of ethyl, allylamine, butyl, diethylaminoethyl, phenyl and allyl derivatives.* Essentially, these derivatives behave similarly to yohimbine HCl but ethyl yohimbine

[1] Meyer, F., *Arch. f. d. ges. Physiol.*, 1912, 223.

[2] Hamet, M., *Comp. rend. Acad. d. sc.*, 1925, **180**, 2074.

[3] Hamet, M., *Comp. rend. Soc. de Biol.*, 1931, **108**, 963.

[4] Weger, P., *Comp. rend. Soc. de Biol.*, 1927, **96**, 801.

* The yohimbine derivatives used in this research were made by Professor David Worral of Tufts College, Medford, Mass.

HCl seems to be the most promising for future studies since it is least toxic.[5]

Attempts at quantitatively determining the effects of the yohimbine derivatives were made by using the method of O. B. Meyer[6] for studying isolated arterial tissue *in vitro* with modifications of Lewis and Koessler,[7] using suspended beef carotid arterial spirals of uniform lengths in the standard Locke oxygenating bath at 37.5°C.

*Results and Conclusions.* This investigation embraces about 60 experiments as represented by a typical tracing (Fig. 1). Epinephrine was effectively constrictor in dosages ranging from 1-200,000,000 and up, but 1-1,000,000 concentration was commonly employed throughout as the standard dosage. The yohimbine derivatives added to the bath after an effective constricting dose of epinephrine exerted no visible response other than nullifying the usual constrictor effect of a second application of epinephrine (Fig. 1B), or the response was in the direction of accelerated relaxation (Fig. 1A). However, after an *exaggerated* constrictor effect of epinephrine as induced by preliminary cocaine application, yohimbine derivatives brought on an accelerated relaxation of the pronounced arterial contraction. This cocaine sensitization of epinephrine activity confirms the well-established work of Froehlich and Loewi[8] and further suggests that epinephrine produces a normal, although diminished, adrenergic response *after* cocaine and yohimbine, because the arterial strip, with its neural components, is *still sensitized* to epinephrine. That is, yohimbine derivatives in the concentrations employed, 1-50,000, do not obliterate cocaine sensitization to epinephrine.

Ephedrine was less responsive as an arterial strip constrictor but its effect also was neutralized by the yohimbine derivatives.

To preclude the probability that "epinephrine reversal" might be due to profound muscular depression by yohimbine derivatives, histamine and barium chloride were added to the bath after yohimbine radicles and their customary constrictor effects observed. Also, sodium nitrite was found to be capable of markedly relaxing strips which had been exposed to the yohimbine derivatives.

Thus the conclusion seems warranted that the yohimbine derivatives, in the concentrations employed in these studies, exhibited no direct muscular effects, and hence that "epinephrine reversals" were due to depression of adrenergic neural components.

5 Young, A. G., *J. Pharm. and Exp. Therap.*, 1935, **54**, 164.

6 Meyer, O. B., *Z. f. Biol.*, 1906, **48**, 352.

7 Koessler, K. K., and Lewis, J. H., *Arch. Int. Med.*, 1927, **39**, 182.

8 Froehlich, F., and Loewi, O., *Arch. Exp. Path. Pharm.*, 1910, **62**, 159.

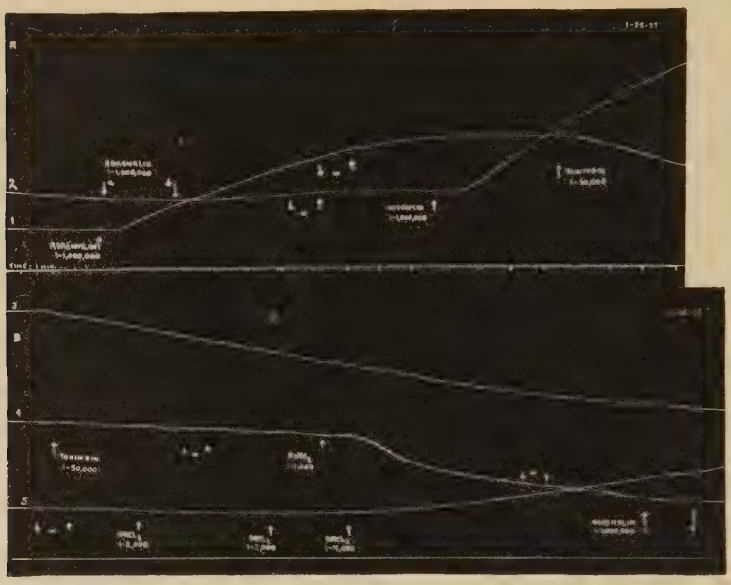


FIG. 1.

Time = 1 minute.
A—No. 1, Adrenalin, 1-1,000,000
Yohimbine, 1-50,000
No. 2, Adrenalin, 1-1,000,000
Histamine, 1-1,000,000
w = washing

B—No. 4, Yohimbine, 1-50,000
Sodium nitrite, 1-1,000
Adrenalin, 1-1,000,000
No. 5, Barium chloride, 1-2,000
" " 1-4,000

*Summary.* 1. The hydrochloride salts of yohimbine derivatives (ethyl, allyl-amine, allyl, butyl, phenyl and diethylaminoethyl) in the dosage used, 1-500,000 to 1-50,000, do not seem to directly affect the arterial muscle strip whether it is in a contracted or relaxed state. 2. A predetermined, consistently constricting dose of epinephrine HCl, 1-1,000,000, or ephedrine, 1-25,000, administered after yohimbine derivatives is inhibited in its action. 3. This inhibitory action of the yohimbine radicle on epinephrine can be obviated by previously sensitizing the arterial strip to epinephrine by addition of cocaine. 4. Musculo-tropic agents such as histamine, 1-1,000,000, barium chloride, 1-1,000, and sodium nitrite, 1-1,000, are not modified in their actions by yohimbine derivatives. 5. These new yohimbine derivatives are antisympatheticomimetic agents.

10395 P

## Bactericidal Effect of an Extract of a Soil Bacillus on Gram Positive Cocci.

René J. Dubos.

*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*

An unidentified spore-bearing bacillus, capable of causing the lysis of living gram-positive cocci, has been isolated from a soil sample to which suspensions of these cocci had been added over a long period of time. Autolysates of cultures of the soil saphrophyte have yielded a soluble factor which lyses living staphylococci, pneumococci (R and S forms, irrespective of type-derivation), hemolytic, green and indifferent streptococci (all types so far tested). The active principle is not volatile, does not dialyze through collodion membranes, and is heat-labile. It is very stable at alkaline reactions, but is rapidly inactivated at reactions more acid than pH 5.5, even at room temperature. When maintained at 0°C, the active principle can be precipitated quantitatively at pH 4.2-4.4, and the precipitate, redissolved in a neutral medium, exhibits the lytic activity of the original solution.

Several tests have been used to investigate the effect of this soluble extract upon the living cells of a number of bacterial species.

1. *Lytic activity.* 1 mg (dry weight) of the bacterial extract, added to $10^9$ pneumococci in a medium at pH 7.5, causes complete lysis of the bacterial cells after 1 hour at 37°C.

Staphylococci undergo solution under the same conditions, but somewhat more slowly. Although lysis of streptococci can also be obtained, it is always less complete and requires larger amounts of extract.

2. *Bactericidal effect.* Pneumococci, streptococci, and staphylococci incubated at 37°C with the extract, are rapidly killed. For instance, 1 mg of extract (dry weight) is enough to kill $10^{10}$ pneumococci or streptococci in 2 hours at 37°C. Similar results have been obtained with staphylococci. The cocci, however, do not lose their viability when the mixtures of extract and bacterial cells is maintained at 0°C even for several hours.

3. *Inhibition of growth.* Minute amounts of extract added to nutrient broth prevent the growth of gram-positive cocci. For instance, 0.0001 mg of extract is sufficient to inhibit the growth of $10^7$ pneumococci inoculated into 5 cc of broth. Inhibition of growth of streptococci and staphylococci requires somewhat larger amounts

of extract. On the contrary, the extract is entirely unable to prevent or retard the multiplication of any of the gram-negative bacilli so far tested, even when the test is carried out with very small inocula (10 bacilli) and large amounts of extract.

4. *Inhibition of dehydrogenase-activity.* Streptococci which have been incubated at 37°C with the bacterial extract, lose the ability to reduce methylene blue in the presence of glucose. In fact, inactivation of the dehydrogenase can be recognized before any morphological alteration of the cocci has taken place; it appears likely, therefore, that lysis is only a secondary process following some injury inflicted upon the cell.

5. *Protective effect against infection of experimental animals.* Intraäbdominal injection of the bacterial extract protects white mice against infection with large numbers of virulent pneumococci. A single injection of 2 mg of the extract will protect white mice against 10,000-100,000 fatal doses of pneumococci of Types I, II, III, V, VIII (other types have not been tested). Protection against larger doses requires repeated treatments. The extract also exerts a curative effect when administered to mice several hours after infection with virulent pneumococci. Preliminary studies carried out with Dr. R. C. Lancefield indicate that the extract is also capable of protecting mice against infection with hemolytic streptococci.

*Discussion.* The results of *in vitro* experiments seem to indicate that the protective action *in vivo* of the bacterial extract is due to a direct bactericidal effect on gram-positive cocci. It is interesting to contrast this direct effect with the mechanism of the protection induced by a bacterial enzyme that hydrolyses the capsular polysaccharide of Type III pneumococcus. As described in earlier studies, this polysaccharidase does not in any way affect the viability of pneumococci; by decomposing the capsular substance, however, it renders the bacterial cells susceptible to destruction by phagocytosis.[1,2] The polysaccharidase does not attack the specific polysaccharides of other types of pneumococci, and consequently it protects only against infection with Type III organisms. On the contrary, the bacterial extract considered in the present paper inhibits the growth of all gram-positive cocci so far tested and exerts on them a bactericidal effect *in vitro;* its protective action *in vivo* has already been established against several different types of pneumococci and hemolytic streptococci. It is worth emphasizing again, however, that the extract does not affect the viability or inhibit the growth of gram-negative bacilli.

[1] Dubos, R. J., and Avery, O. T., *J. Exp. Med.*, 1931, **54**, 51.

[2] Avery, O. T., and Dubos, R. J., *J. Exp. Med.*, 1931, **54**, 73.

10396 P

## Type "B" Anti-influenzal Rabbit Serum for Therapeutic Purposes.

HATTIE E. ALEXANDER. (Introduced by R. McIntosh.)

*From the Department of Diseases of Children, College of Physicians and Surgeons, Columbia University, New York.*

The treatment of influenzal meningitis in children with sera from horses immunized with *H. influenzæ* has, in general, been unsuccessful.

The investigations of Goodner and Horsfall[1, 2, 3] demonstrated the superiority of rabbit over horse antipneumococcal serum. The causes of this superiority were believed to be, first, the smaller size of the anticarbohydrate molecule of rabbit serum, endowing it with greater powers of penetration of the tissues, and secondly, the absence of the prozonal phenomenon[4] or inhibitory mechanism in rabbit serum, when amounts greater than a critical quantity were used.

Rabbit anti-influenzal serum, because of its greater penetrating powers, and some evidence that it crossed the blood-brain barrier, seemed to offer theoretical advantages over horse serum, assuming that the important antibody of the anti-influenzal serum is the anticarbohydrate as in rabbit antipneumococcal serum.

Pittman[5] has brought forth much evidence that there is a parallelism between the antigenic structure of pneumococci and *H. influenzæ*. She demonstrated a capsule on all smooth strains by Muir's capsule-stain and showed that such strains had the power to form soluble type-specific substances. All strains were found to be bile-soluble and therefore apparently produced enzymes as does the pneumococcus.

Because of this similarity, the knowledge derived from the study of pneumococci has been applied in the selection of a suitable antigen for the production of rabbit antibodies for *H. influenzæ*. The strains used were those isolated from cases of influenzal meningitis. All the meningeal strains from children thus far reported have been Type B. Typing was performed by the capsular-swelling

1 Horsfall, F. L., Jr., Goodner, K., MacLeod, C. M., and Harris, A. H., 2nd, *J. A. M. A.*, 1937, **108**, 1483.

2 Horsfall, F. L., Jr., Goodner, K., and MacLeod, C. M., *N. Y. State J. Med.*, 1938, **38**, 245.

3 Goodner, K., Horsfall, F. L., Jr., and Dubos, R. J., *J. Imm.*, 1937, **33**, 279.

4 Goodner, K., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1936, **64**, 369.

5 Pittman, M., *J. Exp. Med.*, 1931, **53**, 471.

method with antisera obtained from Pittman. A large loopful (3-4 mm) of Type B antiserum is placed upon a coverslip. To it is added a 1 mm loopful of dilute young culture or biological fluid containing the suspected *H. influenzæ* organisms. With this a 1 mm loopful of Loeffler's methylene blue is thoroughly mixed. The resulting mixed drop is then suspended over a hollow-ground slide and sealed with cedar oil. The capsules when exposed to type-specific antisera become swollen, opaque, and outlined as do pneumococci typed by the Neufeld method. This hitherto unreported procedure has been useful in the rapid identification of *H. influenzæ* before cultural results are available, and in the determination of the stage at which cultures are presumably most suitable for use as antigen.

For the latter purpose, we have seeded Levinthal broth with 15% of its volume of a 24-hour broth culture, incubated 5 to 6 hours, added formalin to 0.5% and centrifuged, resuspending the organisms in 1/10 the original volume of physiological saline containing 0.5% formalin. Marked capsular swelling could be demonstrated in organisms thus treated. This suspension was used for intravenous injection of rabbits in 3 series of 6 daily inoculations each, with 8-day rest periods intervening. The total quantities of vaccine used in the successive series were 0.5, 0.85, and 1.85 cc respectively. Following the first 6 weeks' schedule as outlined above, the second and third series doses were alternated for 3 months. Subsequently, the third series doses were used every other week during the entire course of injection.

The rather crude test of potency was a determination of the highest dilution of the serum that produced capsular swelling of the organisms. The average titers after 6, 8, 15, 20, and 24 weeks were respectively 1:10, 1:20, 1:40, 1:80, and 1:320. Two determinations were made on each period-sample.

The antiserum produced by this method has yielded encouraging results in the treatment of children with H. influenzal meningitis. Untreated serum was used intrathecally, a globulin-fraction intravenously.